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ANALYSIS OF YIELD OF HARD RED SPRING WHEAT GROWN FROM SEED OF DIFFERENT WEIGHTS AND ORIGIN [1]

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INTRODUCTION

This paper presents data obtained on plantings of wheat of different kernel size. Two experiments were carried out, the first in 1936, when weather conditions were very adverse. Rates of seeding in this experient varied from light to very heavy with some of them similar to those used in farm practice. In the second experiment, in 1938, large seed that had matured in the greenhouse and smaller seed that had matured out of doors were space planted.

REVIEW OF LITERATURE

Many papers have been written dealing with the effect of weight or size of seed upon the resulting crop. As far as the cereals are concerned, most investigators report positive relationship between the crops obtained and the use of heavy and light and large and small parent seeds. Mallach [2] reviewed the literature rather completely and found this to be generally true. In his own experiment with spring wheat and barley the seeds were sorted as to size and then classified according to weight. They were spaced in rows at the rate of about 9 per square foot. Generally the heavier seeds gave the greater grain yield per plant. In 52 comparisons with wheat, the grain yield per plant from the heaviest kernels was 16 percent greater than from the mediumweight kernels. The yield of grain per plant was essentially the same for lightweight and mediumweight kernels. The yield per plant reported by Mallach from the mediumweight and heavyweight seeds are equivalent, respectively, to 60.5 and 70.2 bushels per acre.

Kiesselbach and Helm [3] worked witb spring and winter wheat and with Kherson oats. Spring wheat, seeded in equal number of kernels per unit area, gave 11 percent greater yield per acre from heavy kernels. When equal weights of seed were planted per row, the difference in yield was scarcely appreciable. The yields were generally between 10 and 12 bushels per acre.

[1] Received for publication June 3, 1940.

[2] MALLACH, JOSEF. UNTERSUCHUNGEN ÜBER DIE BEDEUTUNG VON KORNGRÖSSE UND EINZELKORNGEWICHT BEIM SAATGUT. Wiss. Arch. f. Landw., Abt. A, Arch. f. Pflanzenbau 2: 219–295. 1929.

[3] KIESSELBACH, T. A. and HELM, C. A. RELATION OF SIZE OF SEED AND SPROUT VALUE TO THE YIELD OF SMALL GRAIN CROPS. Nebr. Agr. Expt. Sta. Res. Bul. 11, 73 pp., illus. 1917.

WHEAT GROWN FROM LIGHTWEIGHT AND FROM HEAVYWEIGHT SEED

MATERIAL AND METHODS USED

In the spring of 1936 a quantity of wheat which showed marked variation in size of seed was available from the cross Ceres × (Hope × Florence) (breeding number 9.81.45.12). By the use of sieves and by hand picking, two classes of seed grain were prepared, one averaging 26.6 mg. and the other 40 mg. per kernel. The experiment was planned to allow comparisons between rates of seeding differing (1) in number of kernels, with equal seed weights per unit area, and (2) in weight of kernels, with equal numbers of seeds per unit area. The lowest rate of seeding was 11.1 pounds per acre of lightweight seed. As all plantings were made in 8-foot rows, this rate was equal to 0.93 gm. per row of 35 kernels. The next rate was about 50 percent higher, or 54 kernels weighing 1.44 gm. per row, and was essentially the same as the lowest rate for wheat weighing 40. mg. per kernel. The rate of seeding of both classes of wheat was stepped up 50 percent for each increment until 10 rates of seeding were provided for each class. An exception was made between the rates of 406 and 610 kernels per row by the introduction of a rate of 508 kernels, a 25-percent increase in the rate of seeding. See table 1.

TABLE 1.—*Weight of seed grain planted when heavyweight and lightweight kernels were used, mature heads developed per kernel planted, and yields per acre secured when equal numbers of kernels were planted per unit area, using 2 classes of seed grain weighing 26.6 mg. and 40 mg. per kernel.*

[Differences are shown in weight of grain seeded per acre, the number of mature heads per kernel planted, and the yield in bushels per acre]

Item	Data when indicated number of kernels were planted per row										Average
	35	54	80	120	180	270	406	508	610	916	
Lightweight seed planted per acre.....pounds..	11.1	17.2	25.5	38.3	57.5	86.2	130	162	195	292	
Heads per kernel.....number..	3.11	2.71	2.17	1.59	1.26	.98	.86	.84	.82	.75	1.51
Yield, per acre.....bushels..	9.88	12.95	15.55	14.65	15.13	16.25	15.43	15.85	15.25	11.44	14.24
Heavyweight seed planted per acre.....pounds..	17.2	25.5	38.3	57.5	86.2	130	195	244	292	439	
Heads per kernel.....number..	3.33	2.80	2.23	1.84	1.26	1.02	.94	.88	.83	.76	1.59
Yield, per acre.....bushels..	12.13	14.68	16.08	17.28	17.83	17.90	17.65	18.70	15.28	12.45	16.00
Difference:											
In weight of grain seeded pounds..	6.1	8.3	12.8	19.2	28.7	44.0	65.0	82.0	97.0	147.0	
In heads per kernel planted number..	.22	.09	.06	.25	0	.04	.08	.04	.01	.01	.08
In yield per acre...bushels..	2.25	1.73	.53	2.63	2.70	1.65	2.22	2.85	0.03	1.01	1.76

Provision was thus made for 10 comparisons of results obtained from equal numbers of kernels per row and for 8 comparisons from equal weights of seed grain per row. Each planting was repeated 4 times, thus making 80 rows in the experiment proper, and these were randomized in 4 blocks. Each yield row was provided with a pair of guard rows seeded with a drill at the usual rate of 75 pounds per acre. A stand count taken 25 days after seeding showed that 98 percent of the seed had developed plants with essentially no difference in average stand from the 2 weights of seed or from the different rates of seeding.

SEASONAL CONDITIONS

Weather conditions during the growing season as a whole, and especially during July, were by far the worst on record. The excess daily temperature above normal from May through July averaged 7° F., while the daily excess for July was nearly 12°. The rainfall for the 3 months was 21 percent of normal, and for July it was at the record low of 13 percent of normal.

EXPERIMENTAL RESULTS

Table 1 shows the rates of planting, the heads produced per kernel planted, and the yields for the 10 comparisons. Three sets of differences are also shown. Kernels planted per row were the same for the 2 weights of seed.

In all cases the heavy kernels gave yields larger than the light kernels when the results obtained from seeding equal numbers are compared. The average difference in yield of 1.76 bushels is very significant indeed. It is not evident that the continually greater quantities of seed used per acre had any corresponding effect on differences in yield. In all cases the weight of the heavy seed planted was approximately 50 percent greater than that of the light seed. The heads produced per kernel planted are slightly greater for the heavy-kernel seeding; and, while the average difference is but 0.08 head per kernel, this difference is significant with odds of 60 to 1. The significance of these differences is calculated from a total of 79° of freedom with 57 for error.

It is quite evident from table 1 that yields maintain themselves with various rates of seeding to about 200 pounds per acre. Indeed, with the 40-mg. seed the maximum yield resulted from 244 pounds of seed per acre, which is about three and one-fourth times the quantity usually used in farm practice. One might have presumed that such heavy seeding under the adverse weather conditions that obtained would be inimical to good yields, in comparison with lower rates; but it evidently was not.

The comparison of the results obtained from equal weights of seed grain per acre, the number of kernels differing for each weight class, is shown in table 2.

TABLE 2.—*Number of heavyweight and of lightweight kernels planted, mature heads developed per kernel planted, and yields per acre secured when equal weights of seed grain weighing 26.6 mg. and 40 mg. per kernel are planted per unit area*

[Differences are shown of weight of grain seeded per acre, the number of mature heads per kernel planted and the yield in bushels per acre]

Item	Data when indicated quantity of seed was planted per acre								Average
	17.2 pounds	25.5 pounds	38.3 pounds	57.5 pounds	86.2 pounds	130 pounds	195 pounds	292 pounds	
Kernels of lightweight seed planted per row...number...	54	80	120	180	270	406	610	916	
Heads per kernel.....do....	2.71	2.17	1.59	1.26	0.98	0.86	0.82	0.75	1.39
Yield, per acre.....bushels..	12.95	15.55	14.65	15.13	16.25	15.43	15.25	11.43	14.58
Kernels of heavyweight seed planted per row...number...	35	54	80	120	180	270	406	610	
Heads per kernel.....do....	3.33	2.80	2.23	1.84	1.26	1.02	0.94	0.83	1.78
Yield, per acre.....bushels..	12.13	14.68	16.08	17.28	17.83	17.90	17.65	15.28	16.10
Difference:									
In kernels planted per row.....number...	−19	−26	−40	−60	−90	−136	−204	−306	
In heads per kernel planted.....do....	0.62	0.63	0.64	0.58	0.28	0.16	0.11	0.08	0.39
In yield, per acre bushels..	−0.82	−0.87	1.43	2.15	1.58	2.47	2.40	3.85	1.52

On this basis only eight comparisons are possible. Because equal weights of light and heavy seed were planted, some of the rows of lightweight seed planted at the lower rates may have had an advantage in that more kernels were planted per row. On the other hand, for those rows receiving the heavier seeding the greater number of kernels may have resulted in disadvantage due to overcrowding of plants. That overcrowding actually occurred is indicated by the yield differences. With a seeding rate of 17.2 pounds per acre a heavier yield is obtained from the lightweight kernels, whereas the contrary is true when the rate of seeding is 292 pounds per acre. The net gain in yield with the heavier weight of seeding is 4.7 ± 1.2 [4] bushels per acre. In a similar comparison, when the rates of 25.5 and 195 pounds per acre are considered, the net gain in yield with the heavier weight of seeding is 3.3 ± 1.5 bushels per acre. This greater yield from heavy kernels was in evidence and of significance in the three sets of rows planted at rates comparable with field rates, that is, from 38 to 86 pounds per acre, with an average excess of 1.7 ± 0.6 bushels. In these rows seeded at rates comparable with usual field rates, the number of lightweight kernels planted per row was not excessive, and crowding to the detriment of the seedlings and more mature plants could hardly have occurred more than easily may occur in the field. The number of heads produced per kernel planted from the larger, and more thinly planted, kernels was greater by an average of 0.39 when comparisons are made between results obtained from equal weights of seed planted per row. This difference is very significant.

The size of the kernels harvested is of interest in view of the severe seasonal conditions. The results, comparing the rows in which equal numbers of kernels were planted are shown in table 3.

TABLE 3.—*Weight of kernels harvested from 2 sets of plantings from seed of lightweight and of heavyweight kernels, with comparisons between rows planted with equal numbers of kernels*

Item	Data where indicated number of kernels were planted per row										Average
	35	54	80	120	180	270	406	508	610	916	
Weight of harvested kernels from—	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
26.6-mg. kernels	23.7	24.0	25.4	24.6	24.1	25.3	23.8	24.6	24.4	21.4	24.1
40.0-mg. kernels	25.1	24.9	25.8	27.1	27.6	26.2	25.4	24.6	23.7	23.4	25.4
Difference	1.4	.9	.4	2.5	3.5	.9	1.6	0	.7	2.0	1.3

The mean difference in kernel weight from the two crops is 1.3 mg. in favor of the heavy-kerneled plants. The analysis of variance indicates that this is distinctly significant. The difference in kernel weight when a similar comparison is made between equal weights of seed grain planted per row is 1.6 mg., which is likewise significant.

Mallach[5] did not find consistent differences in the kernel weight of grain in plants grown from heavyweight and lighweight seed. He cited two workers who had secured similar results. In the work herein reported the crop secured from the seed planted with heavy kernels had very significantly heavier kernels.

[4] Standard errors are based on the analysis of variance.
[5] MALLACH, JOSEF. See footnote 2.

One explanation for the fact that the heavy-kerneled seed resulted in a crop with heavier kernels might be that the seed used was not homozygous with respect to seed weight and that a genetic separation had been made automatically in separating for kernel size preparatory to seeding. The possibility of this having occurred seems rather remote. Another explanation is possible. In 1934 certain sister selections of the wheat used in this experiment were grown in a yield trial, and it was found that kernel weight increased with yield with a regression coefficient of 0.86, which was significant. If the proper regression formula were used in this case, the change in kernel weight corresponding to the above changes in yield would be 1.5 mg. This difference agrees very well with those shown in table 3. It seems likely, then, that the difference in kernel weight of the crop is to be associated with differences in yield.

DISCUSSION

The data presented show distinct differences in yield in the wheat crop grown from heavyweight and from lightweight kernels. This result was obtained when the same weights of seed grain and also when the same number of seeds was planted per unit area. In the first instance the yield per acre was 10 percent greater from the seed of the heavy kernels and in the other it was 12 percent greater.

The results secured by Kiesselbach and Helm,[6] already mentioned, are very similar as regards spring wheat to those reported here. Yields of spring wheat reported by them were relatively low and were even less than those secured at Fargo in 1936 when the weather conditions were so very adverse. Their results from the use of large and small kernels of winter wheat were less striking. As an average for 4 years the advantage in yield from the larger kernels was only 5 percent with an average of about 35 bushels per acre. Thus, with better conditions, shown by larger yields, the heavy kernels showed less advantage. Mallach [7] states that favorable environmental conditions tend to eliminate differences in plant growth from seeds of different weight. This seems to have held with spring and winter wheat in Nebraska. In the Fargo experiment, with two lots of seed taken from a presumably pure line, it was possible to obtain the rather extreme kernel weights of 26.6 and 40 mg. This disparity in weight of seed kernels in the experiment herein reported is doubtless much greater than could be obtained on the farm by seed-cleaning machinery with a single lot of grain.

WHEAT GROWN FROM SEED MATURING IN THE GREENHOUSE AND IN THE OPEN

In the writer's experience, wheat that has matured in the greenhouse under favorable conditions produces a crop with kernels larger and of finer appearance than wheat that has matured in the open. The question is, how does a crop produced from greenhouse seed differ from one produced from ordinary seed?

[6] KIESSELBACH, T. A. and HELM, C. A. See footnote 3.
[7] MALLACH, JOSEF. See footnote 2.

MATERIAL AND METHODS USED

In the fall of 1937 families of wheat were available from different crosses made in different years. In each of these families a group of hybrid selections was available for experimental use. Seed of a number of these selections within a number of families was planted in the greenhouse. For each group planted in the greenhouse seed of sib selections was held in reserve for planting the following spring. There were 16 hybrid families all told, with 8 hybrid selections of each grown in the greenhouse and seed of 8 sibs held in reserve. In the spring of 1938 16 hybrid selections were thus available for planting from each of the 16 hybrid families. The 2 lots of 8 in each family were presumably of essentially equal genetic value. One lot in each case had been grown an additional generation. The seed which had been held in reserve from 1937 will be designated outside seed. Seed from the plants grown under glass during the winter of 1937–38 will be known as greenhouse seed. Ceres and Thatcher varieties were included as checks, making 18 groups in all. The 16 hybrid families and the 2 varieties will be called families for convenience.

The various hybrid families are found in table 4, with their breeding and their genetic age.

TABLE 4.—*List of wheat hybrids used in an experiment with seed matured out of doors compared with seed matured in the greenhouse*

No.	Hybrid	Origin	Generation
1	3.2.3.30	R.L.625 × CHF 2754	F_6, F_7.
2	3.2.3.33		
3	3.2.3.64		
4	3.2.3.67		
5	3.2.3.85		
6	5.7	Mercury × (H–44 × Ceres)	F_4, F_5.
7	6.19.7	Mercury × (Mercury × H–44 Ceres)	F_3, F_4.
8	6.19.7.29	Mercury × (Mercury × H–44 Ceres) × (625 × 2754)	F_2, F_3.
9	5.9	Mercury × (Reliance × Hope)	F_4, F_5.
10	6.19.9	Mercury × (Mercury × Reliance-Hope)	F_3, F_4.
11	6.19.9.29	Mercury × (Mercury × Reliance-Hope) × (625 × 2754)	F_2, F_3.
12	6.19.6	Mercury × (Mercury × (Comet × (Hard Federation × Hussar)))	F_3, F_4.
13	6.19.6.29	Mercury × (Mercury × (Comet × (Hard Federation × Hussar))) × (625 × 2754).	F_2, F_3.
14	6.19.0	Mercury × (Mercury × (Hope × Marquis)	F_3, F_4.
15	6.19.0.29	Mercury × (Mercury × (Hope × Marquis) × (625 × 2754)	F_2, F_3.
16	6.18–11.29	(Reliance × Reward) × (625 × 2754)	F_2, F_3.

The maturing of larger kernels from wheat grown is the greenhouse under favorable conditions is probably due mainly to cooler temperatures and controlled heat. In half of the greenhouse, where the plants were grown in 6-inch pots, three to the pot, the temperature was allowed to run higher, and as a result the seed was smaller and quite comparable to seed produced in the open. Ceres and Thatcher were grown in the greenhouse to furnish seed for the experiment, but as these plants were also subject to temperatures above the optimum no marked excess in size over the outside seed was in evidence. This was also true for five of the hybrid families grown in pots. As a result, the kernel weight of the greenhouse seed was in a few cases less than that of the comparable outside seed, and in one instance decidedly so. The distribution of excess kernel weight of the seed from plants maturing in the greenhouse over the corresponding outside seed is as follows, expressed as milligrams per kernel.

Excess of seed weight:	*Number of pairs*
−3	2
−2 to +1	3
2 to 5	2
6 to 9	5
10 to 13	4
+14	2

In the three groups with excess kernel weights centering at 3.5, 7.5, and 11.5 mg. the greenhouse seed showed excess weights of 17, 24, and 36 percent, respectively. Thus in most cases there was a rather pronounced difference in weight between the two classes of seeds.

The seeds were space-planted 2 inches apart in unguarded rows 8 feet long and 12 inches apart. The seed, previously taped in the laboratory by the device invented and constructed by Glenn Smith,[8] was planted in tape form with a Columbia drill, modified by Smith for the purpose. This method greatly facilitated space planting. The 36 planting units each had 8 subunits, as indicated above. The setup of the experiment was a 6 by 6 square lattice, following the plan of Yates,[9] with 4 sets of *X* and 4 of *Y* blocks. The outside seed of 3.2.3.30, for example, consisted of 8 selection units, 7, 8, 10, 11, 16, 22, 34, and 41, of which 4 were in *X* blocks and 4 in *Y* blocks.

Seedling counts were taken May 10, when the stand was found to be 96.4 percent of the seeds planted. The stand from the greenhouse seed was 0.2 percent greater than that from the outside seed, a difference which lacked significance. Plants from the greenhouse seed headed 0.2 of a day earlier than those from the outside seed, a difference which was just significant.

The plants were pulled at harvesttime, the two end plants of each row discarded, and the remaining plants wrapped in a bundle and laid away until a study could be made. At that time the plants and the fertile culms were counted, and a random sample of 50 heads were taken, omitting any heads obviously defective. Notes were also taken as to disease present. (Rust notes had been taken previous to harvest.) Occasional plants killed by root rot were discarded. In general, rust was of little moment as most of the wheats were resistant. Exceptions were Thatcher, attacked by leaf rust, and Ceres and the hybrid 6.18–11.29, attacked by stem rust. The bundles were threshed in a small machine which allowed all seed to be saved. The 50-head samples were weighed and counts were made of total spikelets, fertile spikelets, sterile spikelets, and three-kerneled spikelets. The wheat from the 50 heads was weighed, the kernel weight was taken, and an estimate of kernels per head was made. The above work was done for each of the 288 bundles.

In calculating bushels per acre the rows were estimated at full stands of 48 plants per 8 feet. The occasional missing plants had little influence upon yield as the correlation coefficient calculated between plants per row and yield was found to be 0.14. Omitting Ceres and Thatcher, the varieties having the fewest plants per row, the coefficient was −0.04. If differences in stand had influenced yield per plant in such a way that fewer plants per unit area had developed larger individual yields then a significant negative correlation coefficient would have been expected.

[8] SMITH, GLENN S. GUMMED-PAPER TAPE FOR SPACE-PLANTING WHEAT. Amer. Soc. Agron. Jour. 30: 348-352, illus. 1938.

[9] YATES, F. A NEW METHOD OF ARRANGING VARIETY TRIALS INVOLVING A LARGE NUMBER OF VARIETIES. Jour. Agr. Sci. [England] 26: [424]-455. 1936.

EXPERIMENTAL RESULTS

Effect of Kernel Size Upon Yield and Other Characters

The average yield for the entire experiment was 42.7 bushels per acre, with a standard error for a single yield of 12.9 percent of this mean, using 8-foot rows. The effect of excess size of seed upon the yield was calculated from a comparison of the 2 sets of yields. If all yields are brought into the comparison, some are included which were secured from parent greenhouse seed with a kernel weight equal to or less than the kernel weight of the outside seed, as explained previously. Also, with the Ceres variety the crop was seriously affected with stem rust, which might easily nullify any parental effect of size of seed. In making the yield comparisons the square lattice lay-out was treated as an arrangement of ordinary randomized blocks or replications. When all the 288 samples are used in the analysis of variance, the yields from the seed maturing in the greenhouse and that maturing outside were 43.2 and 42.1 bushels, respectively. The difference required for significance is 1.4 bushel. With the omission of the Ceres variety and the 5 additional families, including Thatcher, the parent greenhouse kernels of which weighed less than, or essentially the same as, those of the outside seed, another calculation was carried through. The family 6.18–11.29 was retained, as it had suffered only moderate rust injury. The number of samples used was 192. The five families were omitted to allow a comparison between plants grown from seed clearly separated as to kernel size, grown in the greenhouse and out of doors. In this comparison seed from the greenhouse plants weighed 9.7 mg., or 31 percent, more per kernel than did that grown outside. The two yields, from the greenhouse and from the outside seed, are 45.33 and 43.36 bushels per acre, respectively, with a significant difference in yield of 1.97 ± 0.86 bushels.

The correlation existing between the excess kernel weight of the greenhouse over the outside seed and the corresponding excess of yield was calculated, omitting the six families indicated above. The r value was found to be 0.08, which is not significant.

The means of various other characters calculated for the two parts of the experiment are to be found in table 5 along with differences and the difference necessary for significance.

TABLE 5.—*Means of various characters calculated from 2 lots of wheat, planted from seed grown in the greenhouse and from seed grown out of doors, with differences and with differences necessary for significance*

[96 pairs of parents are compared]

Character	Greenhouse	Nursery	Difference	Difference necessary for significance
Stand of seedling plant ..... number	46.5	46.2	0.3	+0.5
Time of heading in June ..... date	23.7	24.1	−.4	.3
Plants per row ..... number	44.1	43.8	.3	.7
Heads per plant ..... do	5.0	4.9	.1	.2
Fertile spikelets per head ..... do	12.8	12.7	.1	.1
Sterile spikelets per head ..... do	2.3	2.3	.0	.1
Kernels per head ..... do	27.0	26.4	.6	.7
Spikelets with third kernel ..... do	2.6	2.3	.3	.3
Weight of seed in 50 heads ..... grams	46.6	45.9	.7	1.7
Weight of kernels ..... milligrams	35.4	35.7	−.3	.7
Weight per bushel ..... pounds	58.2	58.2	.0	.3
Weight of 25 straws ..... grams	4.3	4.1	.2	.1
Yield per acre ..... bushels	45.3	43.4	1.9	1.8

Two of the differences just reach the threshold of significance, while three are really significant. The difference in yield is indicated by odds of 47:1; the difference in date of heading has odds of 370:1; while for the weight of straw the difference has odds of 730:1.

A Study of the Hybrid Groups

In order to study the 18 families—the 16 hybrid families and the 2 varieties—the paired means of the various characters studied were combined. In table 6 these families are shown with their character means, their various rankings, the values of the significant differences, and their placements as to rank.

In the study discussed above the 144 pairs of selections were treated as a general population. With such diverse breeding as is indicated in table 1 it seems obvious that the families may properly be considered as units to be compared with one another.

The three wheats attacked by rust show relatively low yields. The low yield of Thatcher is less easily understood than that of Ceres and the hybrid family 6.18–11.29, even with Thatcher's incidence of leaf rust; and a discussion of its behavior will be considered later.

Calculation of Yields

If the stand, the heads per plant, the kernels per head, and the weight per kernel are available, the yield per acre can be estimated. When this was done, using the data in table 6, the differences in yields obtained from threshing the crop and those derived by calculation averaged but 0.3 ± 0.14 bushel. An average change of kernel weight of less than 0.1 percent would have resulted in essentially no difference between the two sets of yields. This close approximation is not surprising as the heads per plant and kernels per head were estimated from the results of the entire row. The weight per kernel was estimated from the sample from 50 heads.

Correlation and Regression Studies

Correlation coefficients were calculated, using the characters of yield, fertile heads per plants, kernels per head, and weight per kernel. This was done when the total of 288 units was used and also when the 3 low-yielding wheats, Thatcher, Ceres, and 6.18–11.29, were omitted, in which case there were 240 units used. These 3 wheats were all injured by rust. The kernel size of Thatcher was quite certainly reduced by leaf rust, and stem rust shriveled the kernels of the other 2. Partial coefficients were calculated in addition to the simple ones. The coefficients are given in table 7.

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TABLE 6.—*Means of the indicated characters obtained from seed matured in the greenhouse and in the open, grown in series 481 in 1938*

[The hybrid selections from greenhouse and outside seed are here averaged to form the 18 family units]

No.	Key	Hybrid or variety	Yield per acre		Weight of seed 50 heads		Weight of 25 straws		Weight of 1,000 kernels		Weight per bushel		Fertile spikelets per head		Sterile spikelets per head		Heads per plant		Kernels per head		Spikelets with third kernel		Stand May 13		Days to heading in June	
			Bushels	Difference	Grams	Rank	Grams	Rank	Grams	Rank	Pounds	Rank	Number	Rank	Number	Rank	Number	Rank	Number	Rank	Number	Rank	Number	Rank	Average number	Rank
1	23	3.2.3.85	51.8		45.7	8	4.12	13	34.8	13	58.4	8	12.7	11	2.4	16	5.5	3	28.3	1	2.9	3	46.9	4	23.0	5
2	35	6.19.6	51.1	0.7	50.7	1	4.34	6	36.7	3	55.8	17	12.9	2	2.3	10	5.7	1	25.6	15	1.8	12	46.5	9	23.0	6
3	21	3.2.3.67	49.8	1.3	44.4	11	4.51	1	35.5	10	57.3	13	12.6	13	2.3	11	5.5	4	27.0	5	.9	16	46.0	13	24.3	15
4	15	3.2.3.64	47.0	2.8	45.3	10	4.27	8	36.7	4	58.3	9	12.7	8	2.0	4	5.0	8	26.7	9	2.2	9	46.6	7	22.4	2
5	55	6.19.9.29	46.6	.4	47.9	5	4.24	11	37.1	2	58.5	5	12.7	12	2.2	8	5.0	9	26.8	8	2.5	7	46.7	5	23.7	10
6	31	5.7	46.5	.1	44.0	13	4.36	4	34.2	15	58.7	3	12.7	10	2.1	6	5.1	6	28.1	2	2.7	6	46.3	10	24.1	13
7	51	6.19.6.29	45.5	1.0	47.5	7	4.46	2	36.6	6	57.9	12	12.7	5	2.3	12	4.9	10	26.5	10	2.9	4	45.6	14	22.5	3
8	41	6.19.7	45.2	.3	49.4	3	4.27	9	36.7	5	59.3	1	13.1	1	2.3	14	4.7	15	27.0	6	3.0	2	45.1	18	24.1	12
9	11	3.2.3.30	44.6	.6	43.4	14	4.02	14	35.3	11	58.1	11	12.4	15	2.5	17	5.1	7	26.1	13	1.6	14	46.1	11	23.2	7
10	53	6.19.7.29	44.4	.2	50.7	2	4.38	3	38.3	1	59.0	2	12.9	4	2.3	13	4.8	13	25.3	18	3.3	1	46.6	8	23.6	8
11	45	6.19.0	43.1	1.3	44.3	12	4.31	7	34.4	14	58.1	10	12.7	7	2.2	7	4.9	12	27.7	3	2.2	11	45.6	15	24.7	16
12	43	6.19.9	42.9	.2	47.8	6	4.23	12	36.4	7	58.5	6	12.9	3	2.2	9	4.6	17	26.8	7	2.2	10	46.1	12	24.9	17
13	13	3.2.3.33	42.5	.4	42.5	15	4.00	15	35.9	9	57.1	14	12.4	16	1.8	3	4.8	14	26.3	11	.5	18	46.7	6	24.2	14
14	33	5.9	40.4	2.1	45.5	9	4.26	10	35.1	12	58.6	4	12.7	6	2.5	18	4.7	16	25.8	14	2.3	8	47.0	3	26.3	18
15	61	6.19.0.29	39.5	.9	48.3	4	4.36	5	36.3	8	58.4	7	12.7	9	2.3	15	4.4	18	25.4	17	2.9	5	47.1	2	23.8	11
16	63	6.18–11.29	34.5	5.0	34.3	16	3.70	16	28.7	16	56.8	15	12.5	14	2.1	5	4.9	11	25.5	16	1.2	15	47.6	1	22.6	4
17	25	Thatcher	28.8	5.7	23.7	17	2.96	18	20.6	17	55.8	16	11.6	18	1.8	2	5.5	2	26.2	12	.5	17	45.4	16	21.8	1
18	65	Ceres	24.7	4.1	16.7	18	3.59	17	17.2	18	48.8	18	12.2	17	1.5	1	5.3	5	27.7	4	1.6	13	45.2	17	23.7	9
Mean of character			42.7	----	42.9	----	4.13	----	33.6	----	57.4	----	12.6	----	2.2	----	5.0	----	26.6	----	2.1	----	46.3	----	23.7	----
Significant differences			4.4	----	3.7	----	.32	----	2.0	----	.7	----	.35	----	.2	----	.5	----	1.6	----	.5	----	.38	----	.6	----
Fall between ranks [1]			3/4	----	7/8	----	12 13/13	----	8/8	----	1/8	----	4/5	----	11 11/12	----	5/6	----	9 1/10	----	5/6	----	1/2	----	1/2	----

[1] Except for yield, the values are not in order of size. The reading of 7/8, for example, indicates that all values below 47.5 g., of rank 7, are significantly lower than the high value of 50.7 g.

TABLE 7.—*Simple and partial correlation coefficients of 15 hybrid families of wheat and also these plus Thatcher, Ceres, and 6.18–11.29*

Characters involved [1]	Correlation coefficient for—		Characters involved [1]	Correlation coefficient for—	
	15 families	15 families plus Thatcher, Ceres, and 6.18–11.29		15 families	15 families plus Thatcher, Ceres, and 6.18–11.29
12	0.67	0.35	13.4	0.65	0.57
13	.50	.36	14.2	.36	.81
14	.08	.65	14.3	.26	.74
23	.07	.06	12.34	.86	.83
24	−.27	−.25	13.24	.80	.76
34	−.28	−.12	14.23	.70	.92
12.3	.74	.35	23.4	−.01	.04
12.4	.72	.70	24.3	−.26	−.25
13.2	.62	.36	34.2	−.27	−.10

[1] Code: 1, Yield; 2, heads per plant; 3, kernels per head; 4, weight per kernel.

Any coefficient in table 7 with a value of 0.25 or more is highly significant. The varieties Thatcher and Ceres have normally smaller kernel weights than components of the hybrid families. The kernel weights of these two varieties, and also of 6.18–11.29, were reduced from their normal by leaf rust or stem rust and yields were thus lowered. Because of this the correlation between yield and kernel weight for the 18 groups is decidedly higher than when these three units are omitted—the value of 0.65 compared with 0.08. The contrary is the case in the two correlations of yield and heads per plant for the Ceres and Thatcher varieties carried more heads per plant than the average. Also, the range in kernel weight is narrowed to about 4.1 mg., the maximum weight lying at 12 percent above the low value of 34.2 mg.

The correlation between heads per plant and weight of kernel is negative, −0.27, and highly significant. The values are essentially the same when Thatcher and Ceres are included. Removing Thatcher, Ceres, and 6.18–11.29, with their lightweight kernels, did not modify the correlation. This was evidently due in part to the fact that these wheats average high in heads per plant. The values are unchanged when kernels per head are held constant. The three characters primarily affecting yield do not have positive correlation with each other. Apparently it is by reason of this that the partial correlation coefficients between yield and the other characters take on such high values.

In the partial correlations calculated from the 15 families, those in which yield is correlated with heads per plant are larger than the other coefficients. It will be shown later in the discussion of partial regression coefficients that this relation was the most effective. A change of a unit in number of stools obviously affects the weight of grain per plant more than does a unit change in weight of kernel.

In the work by Bridgford and Hayes,[10] correlations similar to those considered here are given and a comparison will be of interest. Their terminology is modified to correspond to the writer's notation. Heads per row was used instead of heads per plant but the two characters probably correspond very well. In addition, Bridgford and Hayes used the three characters of plumpness (5), date of heading (7), and

[10] BRIDGFORD, R. O., and HAYES, H. K. CORRELATION OF FACTORS AFFECTING YIELD IN HARD RED SPRING WHEAT. Jour. Amer. Soc. Agron. 23: 106-117. 1931.

height of plant (8) in their calculations. These characters were not used in the work herein reported. The number of pairs used by Bridgford and Hayes in their correlation work was 61. The more interesting comparisons and values are shown in table 8.

TABLE 8.—*A comparison of partial correlation coefficients secured at Fargo, N. Dak., and by Bridgford and Hayes at Morris, Minn.*

Characters involved [1]	Values of Bridgford and Hayes [2]	Values in the present study based on —	
		15 families	18 families
12.34	0.62	0.86	0.83
13.24	.58	.80	.76
14.23	.56	.70	.92
12.34578	.02		
12.578	.04		
13.24578	.09		
13.578	.14		
14.23578	.22		
14.578	.23		

[1] Code: 1, Yield; 2, heads per row (or per plant); 3, kernels per head; 4, weight of kernel; 5, height of plant; 7, plumpness of kernel; 8, date of heading.

[2] These coefficients, except those of the fifth order, were calculated by the author from the zero-order coefficients of the original paper.

The coefficients from Bridgford and Hayes which can be compared with those of the present study, with two characters held constant, are uniformly lower. This may perhaps be attributed in part to the presumable lack of complete uniformity of stand in the Minnesota work with grain seeded thickly in rows. Compensations could not be made for differences in germination, for instance, which would likely be found from row to row when space-planting was not used. As has already been pointed out, the three characters, heads per plant, kernels per spike, and weight per kernel, are responsible for yield per plant; therefore, yield per unit area can be calculated when stand of plants is known. These three characters may be considered primary in determining yield while other characters are secondary in influence. Some other character may modify yield, such as date of heading, but only because one of the three primary characters mentioned is affected. The three coefficients from Bridgford and Hayes with five characters held constant are significantly lower than the corresponding coefficients with two of the primary yield characters held constant; for example, 0.02 is to be compared with 0.62. The mean of the coefficients when only the primary yield factors are concerned is 0.59 and only 0.11 when five factors are held constant. The correlation coefficient is calculated for yield and a factor primarily affecting yield while three factors secondarily affecting yield are held constant. This coefficient is then compared with one obtained when two factors primarily affecting yield are interposed into the group of three just indicated. The effect of this interposition is essentially nil. With the three secondary factors omitted from the group of five, the coefficient becomes highly significant.

Partial regression equations were calculated, two factors being held constant in each of the three cases. The regression coefficients and other values are shown in table 9.

TABLE 9.—*Partial regression equations of yield on heads per plant, kernels per head, and weight per kernel, using 288 pairs of values with 18 families of wheat and 240 pairs with 15 families*

[2 characters are held constant in each instance. Maximum and minimum yields and ranges are given corresponding to the extremes of the independent variables. All coefficients are highly significant.]

Basis of comparison and number of pairs	Regression of yield	X	Y	Difference in—	
				Heads per plant, kernels, or kernel weight	Bushels
Heads per plant:				*Number*	
240	$Y=7.43x_3+8.38$	4.4 5.7	41.1 50.7	1.3	9.6
288	$Y=7.08x_3+7.20$	4.4 5.7	38.4 47.6	1.3	9.2
Kernels per head:					
240	$Y=1.62x_3+2.35$	25.3 28.3	43.3 48.2	3.0	4.9
288	$Y=1.58x_3+0.80$	25.3 28.3	40.8 45.5	3.0	4.7
Weight per kernel:				*Milligrams*	
240	$Y=1.17x_4+3.26$	34.2 38.3	43.3 48.1	4.1	4.8
288	$Y=1.18x_4+2.82$	17.2 38.3	23.1 48.0	21.1	24.9

Like the coefficients of correlation shown in table 7, the regression coefficients with two characters held constant show no marked differences when comparisons are made between the 2 sets of families. Ceres and Thatcher and the family 6.18–11.29, all injured by rusts, were included in the 18-family group. The regression of yield on heads per plant is 7.43 bushels for the 15 families and this is significantly larger than the corresponding coefficient of 5.46 bushels. It is evident that an increment of 1 head per plant would modify the yield more than an increment of 1 mg. in kernel weight, and this fact is expressed in the relative size of the coefficients. It is obvious that the marked regression in yield on kernel weight in the one instance is due to the low kernel weight of Ceres, Thatcher, and 6.18–11.29. The predicted increase of 24.9 bushels would have been less with a normal development of the Thatcher kernel, but it likely would have remained of considerable moment.

From various estimates it was thought that the Thatcher yields in other experiments in 1938 at the North Dakota Agricultural Experiment Station and elsewhere were not cut much more than 25 percent by leaf rust; but, if this was true in the present experiment, the proper yield of Thatcher would have been between 37 and 38 bushels, which is still significantly below that of the better yielding wheats. It is doubtful whether leaf rust lessened the number of kernels per head, as there were five families not affected appreciably by leaf rust that had fewer kernels per head than Thatcher.

The weight of kernel of Thatcher has been obtained in 71 experiments from 1937 to 1939 at various points in North Dakota. In 5 of these the kernel weight was as high as 28 mg. and averaged 28.7 mg., the same as the kernel weight of 6.18–11.29. If the Thatcher kernel in the present experiment had had this weight, the yield would have been estimated at 39.7 bushels per acre, which is 11.2 bushels per acre below the average of the 3 highest yielding families. As the number of kernels per head for Thatcher is but 0.8 below the average of the

3 highest wheats, this factor does not explain the low yield for the variety.

As the difference between the top-yielding families and Thatcher seems to be greater than might be expected under field conditions, even with the modification suggested, the stand of plants may be considered as being below the level necessary for an optimum yield for the variety. If the Thatcher yield were to be readjusted to equal the maximum yields, its stand would be increased by nearly 30 percent to result in about 62 plants per row instead of the 48 used. If such an increase in stand were to result in a direct increase in yield, the plants could not suffer any decrease in heads per plant, although such a decrease would certainly arise if the stand increase were to be continued. The question arises, if the stand of Thatcher had been such as to produce its maximum yield, assuming its yield was not maximum, would the yields of the 3 top families have been augmented through a corresponding increase in their rate of seeding? Do the families with the high yields shown in table 6 represent the absolute maxima for the environment of 1938? They probably do not, and it remains a moot point whether the relatively low average yield of Thatcher in this experiment, brought about in part, as we have seen, by low kernel weight, could have been made equal to the high yields of some of the families by a sufficiently thicker planting.

The multiple-regression equation of estimate was calculated. For the 240 pairs this is—

$$Y=7.43 \text{ heads per plant}+1.62 \text{ kernels per head}+1.17 \text{ kernel weight}-76.8.$$

If one supplies the minimum and maximum family means, the extremes in yield become 36.9 and 56.2 bushels per acre. The smaller yield is but 2.6 bushels below the mean yield of family 6.19.0.29, while the larger yield is 4.4 bushels above the mean of the high-yielding family 3.2.3.85.

DISCUSSION

In the first experiment, carried out in 1936, it was shown that yields were increased by 10 or 12 percent when seed of heavy kernel weight was used in comparison with those from lightweight kernels. In the experiment of 1938 the heavy-kerneled seed resulted in an increased yield of but 4.5 percent, which was a significant value. In the first experiment the heavy kernels outweighed the lighter kernels by 50 percent and in the second by 31 percent. The greater percentage difference in yield in the first experiment must have been due in part to the greater disparity in size of seed. But it is possible that the severity of the season of 1936 might have played some part in emphasizing the lower yields from the lighter kernels. However the differences in the two experiments are rather small and must be considered in connection with their standard errors. Such small differences partake of significance only when many replications enter into the means. It is interesting that in the experiment of 1938 the measurement of two characters taken early, completeness of stand and earliness of heading, showed smaller differences in respect to the standard error than did the yield. One might expect the contrary to hold, under ordinary weather conditions; and Mallach [11] cites

[11] MALLACH, JOSEF. See footnote 2.

several workers who reported that plants grown comparably from heavy and from light seeds and showing measurable differences in size when young tended to lose this difference as they approached maturity.

In the experiment of 1938 the selections within a family or variety were combined to allow a study of yield as modified by characters of the plant. While size of kernel used in planting had at best only a minor effect upon yield, kernel size of the harvested plant did have a very significant influence. The two varieties Ceres and Thatcher and a hybrid family had relatively low yields because of small kernels. The kernels of Ceres and Thatcher are normally of lighter weight than those of the hybrid families, and rust attacks increased the normal disparity in weight. It was shown that even if the Thatcher variety had had a kernel weight of 28 mg. which is relatively high for that variety, the resulting yield would have been equivalent to about 39 bushels per acre, and still very significantly below the average of the hybrid families. The partial regression coefficient expresses more technically what has already been shown, that kernel weight of the harvested crop is a very important factor in influencing yield in this space-planted experiment. If leaf rust had not injured the Thatcher plant, more light would have been thrown upon the question as to whether a small-kerneled wheat plant can compete successfully in yield with one having large kernels when space planting is used. Reference to table 6 shows that the better yielding families are in general above the average for the three characters, heads per plant, kernels per head, and weight per kernel. This would be expected, from the relation of these characters to yield.

The 3 high-yielding families of table 6 had means for the 3 characters just mentioned of 0.6, 0.4, and −0.3 above or below the respective means of the 15 hybrid families. When these differences are applied to the 3 partial regression coefficients applicable to the 15 families, the 2 advances in yield are 4.5 bushels per acre for the excess in heads per plant, 0.6 bushel for kernels per head, and 0.4 bushel decrease for weight per kernel. Thus certainly some of the high yields shown in table 6 may be ascribed mainly to relatively heavy stooling, which also characterized Thatcher, and Ceres to a lesser degree. A kernel weight for Thatcher of 28 mg. is about as high as could be expected, a kernel weight which would place the Thatcher yield well below the average for the 18 groups. Thus, as was indicated earlier in the paper, an increased stand would be necessary to bring the yield of Thatcher up to the higher levels of yield. And here 2 things must be considered. Would the Thatcher plants in a theoretically optimum stand, or one increased by perhaps 30 percent over that actually obtained, carry as many heads per plant as did the plants produced in the nursery? They probably would not. Would the high-yielding families seeded at correspondingly higher rates show correspondingly higher yields? Only when these questions are answered will it be possible to learn the maximum yielding capacities of the 2 classes of wheat, small-kerneled and large-kerneled, with approximately the same stooling capacity, when grown space-planted. To enhance the comparison, plantings could be made also at field rates. More satisfactory conclusions might then be obtained.

While it was shown that the three characters, heads per plant,

kernels per head, and weight per kernel, with a uniform stand are responsible for nearly all of the variability associated with yield under the conditions of the experiment, this is really not the end of the problem. While yield is important in the choice of a wheat variety, other characters must be taken into account, such as quality and relationship to disease other than stem and leaf rust, which were considered here. The character, strength of straw, has not been discussed as it did not enter into this work, but it is of importance in the choice of a variety. Obviously it should be possible to bring out a group of promising hybrid varieties but with differences among them similar to the differences found in table 6. Discussions similar to those in this paper would then be quite pertinent and would not be subject to the restrictions suggested above. The question could still be raised as to the extent to which the partial regression coefficients calculated from the spaced plantings could be applied to wheats planted at field rates. An experiment with this purpose should determine the differences in yielding capacity between large- and small-kerneled wheats. The value of such an experiment can scarcely be questioned.

SUMMARY

Seed wheat from a presumably pure line was separated into two classes with kernels weighing 26.6 and 40 mg. When comparisons were made between the yields of the two classes seeded by using either the same weight of grain or the same number of kernels per unit area, the heavier kernels showed the larger yield, the average differences being highly significant.

In a second experiment heavy-kerneled seed matured in the greenhouse was grown in comparison with lightweight seed matured out of doors. When only the 12 families which had parent greenhouse seed with kernels heavier than the outside seed were used, the average yield of grain from the heavy-kerneled seed was 1.97 bushels per acre above the yield from the seed maturing outside. This difference was significant and was 4.5 percent above the lower yield. The kernels of the greenhouse seed used in planting weighed 40.7 gm. per 1,000, or 31 percent heavier than the outside seed.

Correlation studies between the three independent characters, heads per plant, kernels per head, and weight per kernel, and the dependent character, yield, showed highly significant zero-order coefficients, except between kernel weight and yield when the three families injured by rust were omitted. All partial correlations of the first and second order were highly significant when yield was used as the dependent variable.

Partial regression coefficients were all highly significant. The regression of yield on heads per plant was nearly 7.4 bushels per acre for each unit change of head, 1.6 bushels for each unit change in kernels per head, and 1.2 bushels for each milligram change in kernel weight in the 15 families not injured by rust.

INFLUENCE OF APHID RESISTANCE IN PEAS UPON APHID DEVELOPMENT, REPRODUCTION, AND LONGEVITY [1]

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INTRODUCTION

The occurrence of aphid resistance in peas (*Pisum sativum* L.) was demonstrated by Searls [3] in 1931 and corroborated by Maltais [4] in 1936. Experiments of both workers were conducted under field conditions. Searls found that aphid numbers increased less rapidly on the Yellow Admiral and Onward varieties than on other strains of peas. He classified such varieties as "uncongenial" or "resistant." His conclusions were based on differences between seasonal curves of aphid increase on the varieties tested. These curves were plotted from data collected by making periodic aphid counts on random plant samples throughout the growing season. Maltais, using the same procedure, tested 21 standard pea varieties over a period of 3 years. He found, also, that varieties differed with respect to velocity of aphid increase, and selected Melting Sugar, Champion of England, and Prince of Wales as the most resistant of those studied. Yellow Admiral and Onward, unfortunately, were not included in these tests.

Plant breeders have become interested in recent years in the possibility of developing new plant varieties resistant to insect attack. Work on aphid resistance in peas has been delayed, however, due to the lack of a simple technique for measuring the resistance of plants within segregating families. The field testing procedure used by Searls and Maltais, while accurate, is not well adapted for use in a breeding program because of the large amount of time and labor required for a reading. It has been found, also, that field data based on yield or on the ability of plants to recover after aphid attack cannot be used safely to measure aphid resistance because of differences in the physiological age of plants at the time of aphid injury.[5]

The present study was begun in 1938 in an effort to learn more concerning the relationship between the pea plant and its aphid enemy. It was hoped that the results might aid in the development of a simple testing procedure of use to plant breeders. This paper presents data secured from two experiments concerned with the influence of aphid resistance in peas upon aphid development, reproduction, and longevity.

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[2] The author wishes to acknowledge the advice and counsel of Dr. E. M. Searls, of the Department of Economic Entomology, and Dr. R. A. Brink, of the Department of Genetics, under whose direction the work was done. The statistical analysis was performed by Dr. C. Eisenhart and Miss Frieda S. Swed of the Agricultural Statistical Service to whom the author is indebted for this portion of the paper.

[3] SEARLS, ED. M. A PRELIMINARY REPORT ON THE RESISTANCE OF CERTAIN LEGUMES TO CERTAIN HOMOPTEROUS INSECTS. Jour. Econ. Ent. 25: 46–49. 1932.

[4] MALTAIS, J. B. RESISTANCE OF SOME VARIETIES OF PEAS TO THE PEA APHID ILLINOIA PISI KALT. Ent. Soc. Ont. Ann. Rpt. (1936) 67: 40–45, illus. 1937.

[5] HARRINGTON, CECIL D., and SEARLS, ED. M. INFLUENCE OF THE PHYSIOLOGICAL AGE OF THE PEA PLANT ON ITS RECOVERY FROM APHID DAMAGE. Jour. Agr. Res. 60: 157–161, illus. 1940.

PROCEDURE

Two varieties of canning peas, Perfection and Pride, were used in the study. The former was selected because of its susceptibility to the pea aphid, the latter because of its partial resistance. Plants were grown in sand, using a complete nutrient solution developed for peas by the Plant Pathology Department of the University. The containers were No. 2. enameled tin cans. The aphids (*Macrosiphum* (*Illinoia*) *pisi* (Kleb.)) were of pure-line origin, the line being obtained by isolating a single agamic insect and its subsequent progeny.[6] Gauze-covered lantern globes were used to confine the insects to the growing tips of the test plants. Support for these globes, as well as for the plants

FIGURE 1.—Testing racks with plants and lantern globes in position.

and their containers, was provided by adjustable testing racks, as shown in figure 1.

The globes were ventilated by electric fans located at the front and along the sides of the testing bench. Currents of air directed over the gauze-covered tops of the globes created a partial vacuum within. This caused fresh air to circulate through screen-covered apertures in the platform upon which each globe rested.

Each experiment included 30 Perfection and 30 Pride plants. Each of these plants was infested with a single reproducing aphid. After 4 hours, however, this aphid and all but one of its progeny were removed. Plants on which no young were produced during this short period were infested with one of the excess young removed from other plants. In

[6] Little evidence has appeared to date which would indicate that more than one strain of the pea aphid exists. Nevertheless, to avoid any genetic heterogeniety which might be present, pure-line insects were used.

this way 60 aphids of uniform age were confined upon the 30 individual plant tips of the two varieties.

Data were taken at 4-hour intervals during the period of aphid development. All insects were examined carefully with a hand lens at each reading to determine whether a new stadium had been reached during the previous 4-hour period. This procedure enabled data to be taken relative to the number of hours required by each of the 60 aphids to pass through each of the four nymphal stadia and to reach reproductive maturity.[7] After reproduction began, the progeny of each aphid was carefully removed and counted every 48 hours. The young were removed to prevent overcrowding of the test plants, and to avoid second-generation reproduction which would have resulted if any of the progeny had been allowed to reach maturity. When emaciation and reduced reproduction indicated that the parent aphids were growing weaker, observations were begun and continued at 24-hour intervals in order to record the day on which each parent aphid died. The experiments, which ran consecutively, were concluded upon the death of the last parent aphid in each. Individual records were kept, all data pertaining to any aphid being recorded under its respective rack and globe number.

EXPERIMENTAL RESULTS

A complete set of data was obtained for the second experiment. All averages, for this reason, are based on the full complement of 30 replicates per variety. While taking data on reproduction in the first experiment, however, 8 parent aphids were dislodged and lost. In addition, failure to find and remove all young resulted in second generation reproduction on 3 plants. Data relative to total reproduction were discarded in these cases. No records of longevity could be obtained for the 8 lost aphids. Furthermore, longevity records for 3 other aphids were discarded because the insects died of causes other than old age. In the first experiment, therefore, the averages of total reproduction for Pride and Perfection are based on records of 24 and 25 aphids, respectively, and the averages of total longevity are based on longevity records of 26 and 23 aphids, respectively. The data are presented in table 1.

STATISTICAL TREATMENT AND INTERPRETATION OF DATA

"Student's" t-test was used in the statistical treatment of the data, the values of t (for 58 degrees of freedom) necessary for significance at the 0.05 and 0.01 levels being 2.002 and 2.664, respectively.

It appears from the two experiments that aphid development on Pride differed significantly from that on Perfection with regard to hours to first moult. The aphids required a greater number of hours on Pride, the values of t being significant at the 1 percent level. This difference persists, as far as time is concerned, to the second, third, and fourth moults, the respective t values all being positive and significant. Under these circumstances a difference is anticipated in total time required from birth to reproductive maturity, with aphids on Pride requiring the greater length of time. This hypothesis is supported by a positive value of t, also significant at the 1 percent level.

[7] The pea aphid, theoretically, is mature as soon as it reaches the last, or fifth, stadium. The insects however, do not begin to reproduce until sometime later.

The length of the first stadium is the time required to reach the first moult. There is a significant difference between varieties in the first experiment with regard to the time spent in the second stadium and in the period between the last moult and reproductive maturity, the two t values being significant at the 5 percent level. Except for these differences, in both experiments the original variation between Pride and Perfection, observed in the time to the first moult, remained essentially the same from then on. In other words, there were no significant differences in time spent in the second, third, and fourth stadia, and in the period just prior to reproductive maturity.

TABLE 1.—*Aphid development, reproduction, and longevity on the Perfection and Pride varieties*

Item	Development period (average hours)										Total young produced	Total life span
	Length of stadia				Fourth moult to reproductive maturity [1]	Period from birth to various moults				Birth to reproductive maturity [2]		
	First	Second	Third	Fourth		First	Second	Third	Fourth			
Experiment 1:	*Hours*	*Hours*	*Hours*	*Hours*	*Hours*	*Hours*	*Hours*	*Hours*	*Hours*	*Hours*	*Number*	*Days*
Perfection	35.0	36.6	40.9	45.3	20.6	35.0	71.5	112.4	157.7	178.3	106.4	29.0
Pride	38.2	38.2	40.9	44.9	22.4	38.2	76.4	117.3	162.2	184.6	93.5	23.9
Difference	3.2	1.6	.0	—.4	1.8	3.2	5.1	4.9	6.5	6.3	—12.9	—5.1
t Value [3]	**5.167	*2.548	.000	.491	*2.032	**5.167	**5.605	**4.705	**4.191	**5.754	**4.435	**5.070
Experiment 2:												
Perfection	33.0	38.6	40.0	51.1	19.8	33.0	71.5	111.5	162.6	182.5	109.7	32.6
Pride	35.0	39.2	40.0	52.8	20.4	35.0	74.2	114.2	167.0	187.4	95.6	25.3
Difference	2.0	.6	.0	1.7	.6	2.0	2.6	2.7	4.4	4.9	—14.1	—7.3
t Value [3]	**3.042	1.167	.000	1.545	.784	**3.042	**4.882	**3.466	**3.084	**3.669	**4.271	**5.470

[1] Total hours between fourth moult and reproductive maturity.
[2] Total hours between birth and reproductive maturity.
[3] For 58 degrees of freedom, t at the 0.05 level = 2.002, and at the 0.01 level = 2.664.
*Indicates a significant difference exists at the 0.05 level. (Odds are 20–1 that difference is not due to chance alone).
**Indicates a significant difference exists at the 0.01 level. Odds are 100–1 that difference is not due to chance alone.

Fewer young were produced on Pride than on Perfection, the difference being significant at the 1 percent level. The total length of life was significantly less on Pride, the t value being significant at the 1 percent level.

A study of data on aphid development brings to light some interesting information regarding the influence of resistance upon aphids reared on Pride as compared with those reared on Perfection. This influence may be described as follows: Resistance slows down the rate of development in all stadia, but the reduction is usually too small to be detected statistically for any one stadium, except the first. The influence is, however, cumulative, increasing gradually until reproductive maturity is reached. This trend is particularly noticeable in the results of the second experiment.

DISCUSSION

Under Wisconsin conditions the pea aphid normally produces 15 to 16 generations each year, which are roughly distributed as follows: 2 to 3 upon the primary hosts (alfalfa) in the spring, 7 to 8 upon secondary hosts (peas) in late spring and summer, and 3 upon the primary hosts again in the fall. Peas are planted in April or early May and are

harvested the last week of June (early varieties) or in early July (late varieties). One aphid generation requires 6 to 12 days depending upon the temperature. When peas are young, low temperatures inhibit aphid development and reproduction with the result that little damage is done. With the higher temperatures of late June, aphid generations follow each other in rapid succession, the population soon reaching sufficient proportions to blast blossoms and young pods or even to destroy the plants. The three principal factors that determine whether a pea field is to be damaged are: (1) Size of original infestation, (2) rate of aphid development and reproduction, and (3) duration of infestation. The grower cannot control the original infestation but use of resistant varieties will slow down the rates of aphid development and reproduction. While it is true that the most resistant varieties known today are but mildly resistant, nevertheless the inhibiting influence that they exert upon aphid build-up during the entire season is very large. In this connection the data presented above can be considered. By computing the differences between Pride and Perfection on the percentage basis, utilizing the data in the three right-hand columns of table 1, it is found that Pride in the two experiments increased the time of development 3.1 percent, decreased reproduction 12.5 percent, and shortened the aphid life span by 20 percent. The cumulative effect of this influence, acting upon 8 successive aphid generations, may explain why the more resistant varieties often produce a crop when susceptible strains succumb.

Lack of a simple and accurate technique for determining the comparative resistance of segregating plants is one of the chief factors which has delayed the production of new pea varieties resistant to the pea aphid. The principal purpose of this work, as mentioned above, was to discover which component of the host-parasite complex could be used to best advantage as a basis for measuring the relative aphid resistance of pea plants. In this respect the data indicate that the 3.1 percent increase in time of aphid development for Pride as compared with that for Perfection is not large enough to justify basing a technique on this phase of the life cycle. This fact becomes clear when it is realized that the known range from maximum resistance to maximum susceptibility in pea varieties tested to date is very small, Pride and Perfection representing almost the extremes. The difference of 12.5 percent in reproduction between the two varieties appears to be large enough to justify further work on this subject. The 20 percent difference in longevity is promising, but unfortunately the use of longevity data in measurement of aphid resistance is out of the question due to (1) the technical difficulty of caring for the progenies, and (2) the long period of time (during which a test must be run) before the test insects die.

SUMMARY AND CONCLUSIONS

Two experiments were run under greenhouse conditions to determine the influence of aphid resistance in peas upon aphid development, reproduction, and longevity. The partially resistant Pride and the susceptible Perfection pea varieties were used. Both experiments include 60 aphids, which were confined individually upon 30 plants of each variety. Analysis of the data obtained leads to the following

conclusions, which should be considered only with reference to the varieties used:

1. Aphid resistance reduces slightly (3.1 percent) the rate of aphid development.

2. Aphid resistance inhibits reproduction to a considerable extent (12.5 percent).

3. Aphid resistance decreases aphid longevity (20 percent).

4. The cumulative effect of resistance acting upon aphid populations throughout the pea growing season may explain why resistant varieties are often able to produce a crop when susceptible strains are destroyed.

5. Data on relative rates of aphid reproduction appear to offer a better possibility for measuring the comparative resistance of pea varieties than data on rates of aphid development or length of life.

BUCKEYE ROT OF TOMATO IN CALIFORNIA[1]

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INTRODUCTION

Although buckeye rot of green tomato fruits (*Lycopersicum esculentum* Mill. var. *vulgare* Bailey), caused by *Phytophthora terrestria*, was described by Sherbakoff (*25*) [2] more than two decades ago, the disease was not known to occur in California, where tomatoes are a major crop, until September 1937 when it was observed by J. B. Kendrick in a 100-acre tomato field near Davis. The rot was confined to fruits on or near the ground following a heavy irrigation. In August 1938, infected green Italian pear-shaped tomatoes were received from Modesto, and the disease, based on symptoms only, was identified as buckeye rot. Two months later, this disease was found to be of major importance in the late crop of green-wrap tomatoes grown at Brentwood.

Buckeye rot of green tomato fruits has consistently been ascribed to *Phytophthora parasitica* by investigators in various states and foreign countries. Some of the reported diagnoses were made, apparently, on symptomatology alone or at least on very limited cultural tests. However, Bewley (*4*), in England, may have been the first to suggest that more than one species of *Phytophthora* may cause buckeye rot, naming *P. cryptogea* in addition to *P. parasitica*. Investigations in California not only support Bewley's contentions, but, in addition, indicate that the disease may be produced by other species in the absence of *P. parasitica*.

DISTRIBUTION OF THE DISEASE

According to Sherbakoff (*25*, *26*, *27*), buckeye rot of tomato fruits was first recognized as a new disease in January 1915 at Goulds, Fla., but presumably had been observed as early as 1911, and occurs along both the east and west coasts. The disease has been reported to the Division of Mycology and Disease Survey, United States Department of Agriculture,[3] from Arizona, Arkansas, Colorado, Florida, Illinois, Indiana, Louisiana, Maryland, Massachusetts, Mississippi, Missouri, New Jersey, New York, North Carolina, Ohio, Tennessee, Texas, Virginia, and West Virginia. It also occurs in Puerto Rico. The first record of the disease from California was given by Ramsey [4] in 1939. In view of its wide geographical range, Weimer (*39*) was undoubtedly correct in stating that "buckeye rot of tomatoes, then, is not restricted to the extreme southern States."

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[2] Italic numbers in parentheses refer to Literature Cited, p. 472.

[3] Letter from H. A. Edson, Division of Mycology and Disease Survey, U. S. Department of Agriculture, dated February 19, 1940.

[4] RAMSEY, G. B. FRUIT AND VEGETABLE DISEASES ON THE CHICAGO MARKET IN 1938. U. S. Dept. Agr. Bur. Plant Indus., Plant Disease Rptr., Sup. 114, pp. [27]–40. 1939. [Mimeographed.]

The disease has also been reported in Argentina (*5*), Australia (*14*), the British West Indies (Trinidad, St. Vincent, and Montserrat) (*1*), Canada (*7*), Denmark (*10, 37*), England (*2, 3, 4, 15, 16, 17*), India (*33*), Japan (*29*), Mexico (*28*), the Netherlands (*18*), Palestine (*20*), and the Union of South Africa (*34, 35*).

Further records, which may possibly relate to buckeye rot, occur in the literature and are briefly mentioned in order to make this paper as inclusive as possible. A rot of green tomato fruits, presumably caused by *Phytophthora terrestria*, was found in Cuba in 1918 by Bruner, according to Weimer (*39*). In 1932, just 7 years prior to the first definite record of the disease in the British West Indies (*1*), Briant (*6*) found that an unnamed species of *Phytophthora* was responsible for a fruit rot of tomatoes in Trinidad. In Bermuda, a species of *Phytophthora* belonging to the *P. palmivora* group, was believed by Waterston (*36*) to be the cause of a rot of green tomato fruits (varieties Marglobe, Pritchard, and Break o'Day).

Although the disease has not been reported from Greece and Italy, nevertheless it is significant that Sarejanni (*24*) and Goidanich (*9*) have recently described a collar rot of tomato plants caused by *Phytophthora parasitica*. Samuel (*22*) stated the disease has never been observed in South Australia, although the disease is known to occur in the metropolitan area of Sydney, Australia (*14*).

A rot of tomato fruits, caused by *Phytophthora capsici*, has occurred in the Arkansas Valley of Colorado (*23*), but whether the disease is identical with buckeye rot was not indicated. [5]

SYMPTOMS OF THE DISEASE

The symptoms of buckeye rot of green tomato fruits collected in the Brentwood and Stockton sections, two of the four known centers of infestation in California, are identical with the original description as given by Sherbakoff (*25, 26, 27*) and later by Kendrick (*11*), Ramsey and Link (*19*) whose paper is illustrated by excellent colored photographs, Wager (*34, 35*), Weber and Ramsey (*38*), and Young, Harrison, and Altstatt (*40*). Green fruits of all sizes, if in contact with or near the surface of moist or waterlogged soil, were subject to infection. Irregular-shaped, water-soaked areas, usually but not always at the blossom end of the fruits, constituted the first visible evidence of infection (fig. 1, *A*). As the lesions enlarged, the centers turned brown to blackish-brown and frequently developed either complete or incomplete concentric brown rings (fig. 1, *B, D*). Some fruits were devoid of this ring symptom. On all lesions, however, the advancing margin was water-soaked, while the rate of enlargement was rapid under conditions of high temperature and humidity and an excessive supply of irrigation water. Decayed areas were firm in texture, with little or no aerial mycelium on the surface of the fruits. An abundant mycelial growth developed, however, when diseased fruits were placed in a moist chamber, and the rate of decomposition was accelerated. Lesions on some fruits showed arrested development, the surface tissues becoming hard and dry (fig. 1, *C*).

[5] Since this paper was accepted for publication the following article has appeared: KREUTZER, W. A., BODINE, W. W., and DURRELL, L. W. CUCURBIT DISEASES AND ROT OF TOMATO FRUIT CAUSED BY PHYTOPHTHORA CAPSICI. Phytopathology 30: 972–976, illus. 1940. In this article a field decay of tomato fruits, caused by *Phytophthora capsici*, is described in more detail.

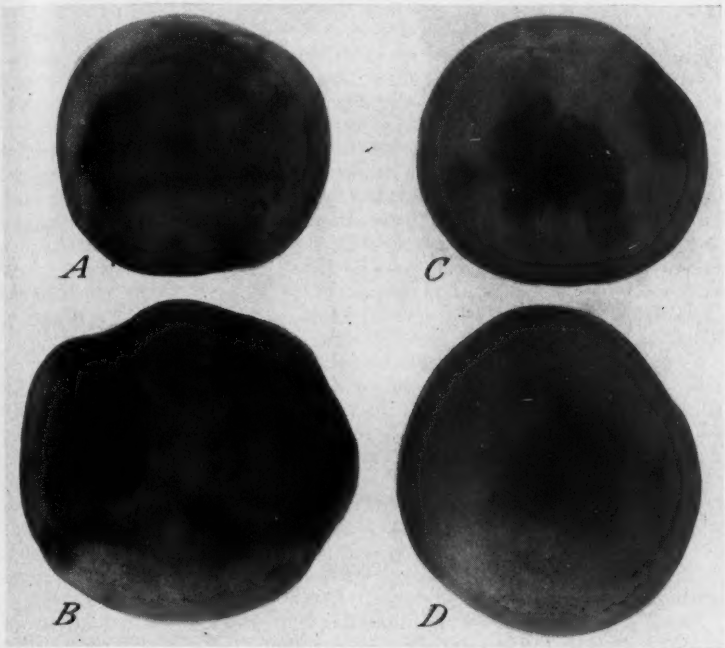

FIGURE 1.—Natural infection of green-wrap tomato fruits grown at Brentwood, Calif.: *A*, Large, irregular-shaped, water-soaked lesion, with brown center; *B*, an aggregate of brown lesions surrounded by a water-soaked margin; *C*, blackish-brown lesions showing arrested development; *D*, water-soaked lesion with brown center and concentric rings.

THE CAUSAL FUNGI, PHYTOPHTHORA CAPSICI AND P. DRECHSLERI

Small tissue fragments were removed aseptically from the advancing, internal margin of lesions of naturally infected, green tomato fruits, planted on malt-extract agar (*13*) in Petri dishes, and incubated at room temperature. Usually, after 48 hours, pure cultures were established on agar slants by transferring hyphal tips from the edge of the colonies. Cultures were made from approximately 300 green tomato fruits in 1938 and 1939.

Although the original cause of buckeye rot was ascribed by Sherbakoff (*25*) to *Phytophthora terrestria*, Tucker (*32*) later determined that the fungus was identical with *P. parasitica* Dast.

Microscopic examination of the California isolates indicated that two species of *Phytophthora* were involved in the rotting of green tomato fruits, namely, *P. capsici* Leonian (*12*) and *P. drechsleri* Tucker (*30*).[6] Cultures of *P. drechsleri* were more numerous than *P. capsici* in 1938, whereas in 1939 all the isolates were referable to *P. capsici*.

[6] An isolate from a rotting tomato fruit collected at Edison, Calif., in November 1940 was identified as *Phytophthora parasitica* Dast. The isolate developed the tufted type of mycelial growth characteristic of *P. parasitica*, formed sporangia and chlamydospores in culture, and exhibited temperature growth relations typical of the species. The isolate was indistinguishable from cultures obtained from tomato fruits from Cuba, Florida, and Missouri.

It is significant that not a single isolate of *P. parasitica* appeared during either season, although this species has heretofore been recognized as the exclusive causal agent of the disease.

The three species have similar temperature-growth relations and may be expected to appear on tomato fruits under similar environmental conditions. *Phytophthora drechsleri* is readily distinguishable from the other species by its nonpapillate sporangia which usually develop sparingly, even when hyphae are transferred to sterile distilled water. *P. capsici* does not develop typical chlamydospores and may thus be distinguished from *P. parasitica*. Furthermore, the sporangia of *P. capsici* are usually somewhat irregular, with a tendency to elongate in the apical region, while those of *P. parasitica* are more frequently of the regular, limoniform type. All three species are variable in production of oogonia, some isolates developing them in fair numbers while in others the sexual stage is never observed.

Tucker (*32*) has shown that the ability to invade and kill stems of pepper plants is a specific character of *Phytophthora capsici*. All isolates from tomato fruits that resembled *P. capsici* morphologically were inoculated into pepper stems; in every instance the fungus invaded and killed the terminal 3 to 6 inches of the stem in 5 days.

Pure cultures of *Phytophthora capsici* and *P. drechsleri*, isolated from naturally infected green tomato fruits collected at Brentwood, Calif., and of *P. parasitica* from an infected tomato fruit collected at Columbia, Mo., were used in the infection experiments. The fungi were grown on malt-extract agar in Petri dishes, incubated at room temperature, and used for inoculum when 4 days old.

Parallel inoculations were made on unwounded, detached, green tomato fruits in the laboratory and on green fruits in situ on healthy tomato plants grown in the greenhouse. In the laboratory, healthy fruits were washed in running tap water, rinsed in distilled water, and dried. A small block of inoculum was placed on the uninjured epidermis and kept moist with absorbent cotton under an inverted preparation dish. In some instances, inoculated fruits were placed in glass moist chambers. No fruits were wounded in any of the inoculation tests. For controls, fruits were handled in the same manner except that sterile agar was substituted for the inoculum.

In the greenhouse, the green fruits on living tomato plants, varieties Sutton's Best of All and Crackerjack, were not washed. The inoculum and moist absorbent cotton were held in place with a rubber band. One to several inoculated fruits were enclosed in a glassine bag after inoculation. A total of 12 fruits were inoculated with each species of *Phytophthora*. Control fruits on living plants were similarly treated except that sterile agar was substituted for the inoculum. All inoculated fruits became diseased, while the noninoculated control fruits remained healthy. The average incubation period was: *Phytophthora capsici*, 7 days; *P. drechsleri*, 11 days; and *P. parasitica*, 7½ days. These artificially induced lesions were identical in color and consistency with those resulting from natural infection. Concentric rings, either complete or incomplete, developed in some lesions (fig. 2, *A*), but usually this symptom was lacking (fig. 2, *B*). It is doubtful whether the zonation said to be characteristic of buckeye rot is a good distinguishing character. In these inoculation tests, it was observed that fruits vary a great deal in the development of the zonate

character, even when infected by the same isolate. The 3 species of *Phytophthora* produced identical symptoms when compared with each other, and it was impossible, therefore, to differentiate between them by using expressed symptoms as criteria. Laboratory tests yielded comparable results, with no essential difference in the incubation periods of the respective species tested. Reisolations were made from all infected fruits, and infection was again obtained with the reisolates.

These tests for pathogenicity indicate that species other than *Phytophthora parasitica* may also cause buckeye rot of tomato, and, in California, they are represented by *P. capsici* and *P. drechsleri*.

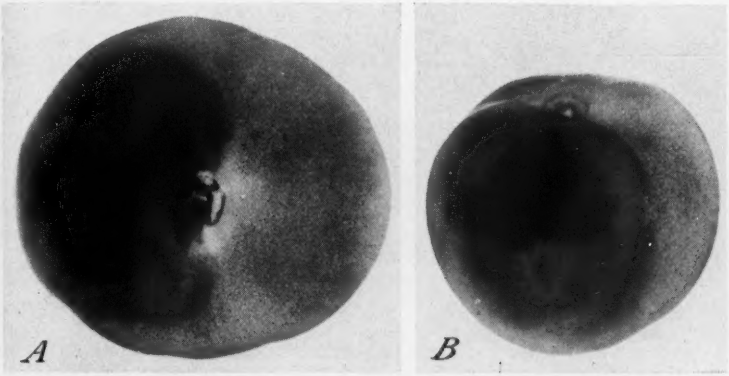

FIGURE 2.—Artificial infection, without wounding, of green Crackerjack variety tomato fruits, in situ on plants grown in the greenhouse. *A*, Large, water-soaked lesion, with slightly darker center, showing concentric ring effect produced by *Phytophthora capsici; B*, large, water-soaked lesion, with blackish-brown center, produced by *P. drechsleri*.

An isolate of *P. capsici* from a naturally infected honeydew melon fruit (*Cucumis melo* L. var. *inodorus* Naud.) has previously been shown to be pathogenic to green tomato fruits (*31*), resulting in lesions typical of buckeye rot.

That green or immature tomato fruits are highly susceptible and may be invaded by *Phytophthora parasitica* in the absence of wounds was previously mentioned by Sherbakoff (*25*), Gardner (*8*), Kendrick (*11*), Ramsey and Link (*19*), Rosenbaum (*21*), Wager (*35*), and Weber and Ramsey (*38*), so that the results presented in this paper are merely confirmatory.

DIFFERENTIAL HOSTS

Parallel inoculations of various fruits, roots, and tubers were made in the laboratory with 4-day-old cultures of *Phytophthora capsici*, *P. drechsleri*, and *P. parasitica* in an attempt to find a differential host which could be used for rapid identification of various isolates from naturally infected green tomato fruits. Infection of ripe tomato, eggplant (*Solanum melongena* L. var. *esculentum* Nees), avocado (*Persea gratissima* Gaertn.), Bartlett pear (*Pyrus communis* L.), and honeydew melon fruits was obtained with the three species of *Phytophthora*, but all produced identical symptoms on these hosts.

However, Yellow Crookneck and Zucchini pumpkin fruits (*Cucurbita pepo* L. var. *condensa* Bailey) were readily infected by *Phytophthora capsici* and *P. parasitica*, but not by *P. drechsleri;* Purple Top White Globe turnip roots (*Brassica rapa* L.) were infected only by *P. drechsleri* and *P. parasitica;* and carrot roots (*Daucus carota* L. var. *sativa* DC.) by *P. capsici* and *P. drechsleri*. *P. capsici* alone caused infection of bell or sweet green pepper (*Capsicum annuum* L. var. *grossum* Sendt.), Newtown Pippin apple (*Pyrus malus* L.), and cucumber fruits (*Cucumis sativus* L.). Thus, for the isolates tested, pumpkin and bell pepper fruits and turnip and carrot roots may be used as differential hosts.

All three species of *Phytophthora* failed to infect Garnet Chili and White Rose potato tubers (*Solanum tuberosum* L.); muskmelon (*Cucumis melo* L.), Klondike watermelon (*Citrullus vulgaris* Schrad.), and lemon fruits (*Citrus limonia* Osbeck); and garden beet roots (*Beta vulgaris* L. var. *crassa* Alef.).

SUMMARY

Buckeye rot of green tomato fruits is prevalent in several of the interior valleys of central California.

The disease is favored by high temperatures and humidity, and the most important factor favoring infection in California appears to be contact of the fruit with moist soil or with irrigation water.

Isolation, inoculation, and taxonomic studies show that the disease in California is caused by *Phytophthora capsici* and *P. drechsleri*, rather than by *P. parasitica*.

Several differential hosts, including pumpkin and bell pepper fruits and carrot and turnip roots, were found. These may assist in the identification of *Phytophthora* species isolated from diseased tomato fruits.

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RING NECROSIS OF CABBAGE[1]

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INTRODUCTION

In 1935 Smith (*4*)[3] described a virus disease of cabbage and brussels sprouts in England, which he designated as "ringspot" because of the characteristic deeply sunken lesions surrounded by almost black necrotic rings. When leaves of young cabbage plants were inoculated, necrosis usually developed on the inoculated leaves and a mosaic mottling without preliminary vein clearing developed in the younger systemically infected leaves. Necrotic and occasionally chlorotic rings followed. In 1938 Tompkins et al. (*9*) described a virosis of cabbage from California under the name of "black ring." The early symptoms on inoculated cabbage (no reaction on the inoculated leaves themselves was recorded) consisted of numerous small chlorotic lesions in which necrotic margins developed commonly after 2 or 3 weeks. Under field conditions the necrotic rings were most conspicuous on the under surface of the older, outer head leaves. It is to be noted that neither mosaic mottling nor vein clearing was described in connection with this disease on cabbage.

In 1937, at Madison, Wis., the writers noted in a plot of cabbage infected with a mosaic disease described elsewhere (*2*) necrotic ring lesions on many mature plants, particularly on the outer head leaves. From such plants a virus was obtained that produced various degrees of chlorosis and necrosis on cabbage. Although it had certain points in common with the diseases noted above, it was sufficiently distinct to warrant the present description. In order to distinguish the disease from others it is designated herein as "ring necrosis" of cabbage. A preliminary report has been published (*3*).

SYMPTOMS AND HOST RANGE

One of the major points of difference between ring necrosis and black ring is the fact that ring necrosis is masked in the greenhouse at 13° to 19° C. The symptoms herein described were observed on plants growing at 22° to 25°.

CRUCIFEROUS HOSTS

The following crucifers were inoculated mechanically with ring necrosis virus, according to methods described in the section on trans-

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[3] Italic numbers in parentheses refer to Literature Cited, p. 491.

mission of the virus, and all were found to be susceptible and to exhibit systemic symptoms.

Brassica oleracea var. *capitata* L. (cabbage, vars. Jersey Queen, Marion Market, Wisconsin All Seasons, Wisconsin Hollander).
Brassica oleracea var. *botrytis* L. (cauliflower, var. Snowball; sprouting broccoli, var. Green Sprouting).
Brassica oleracea var. *gongylodes* L. (kohlrabi, var. Early White Vienna).

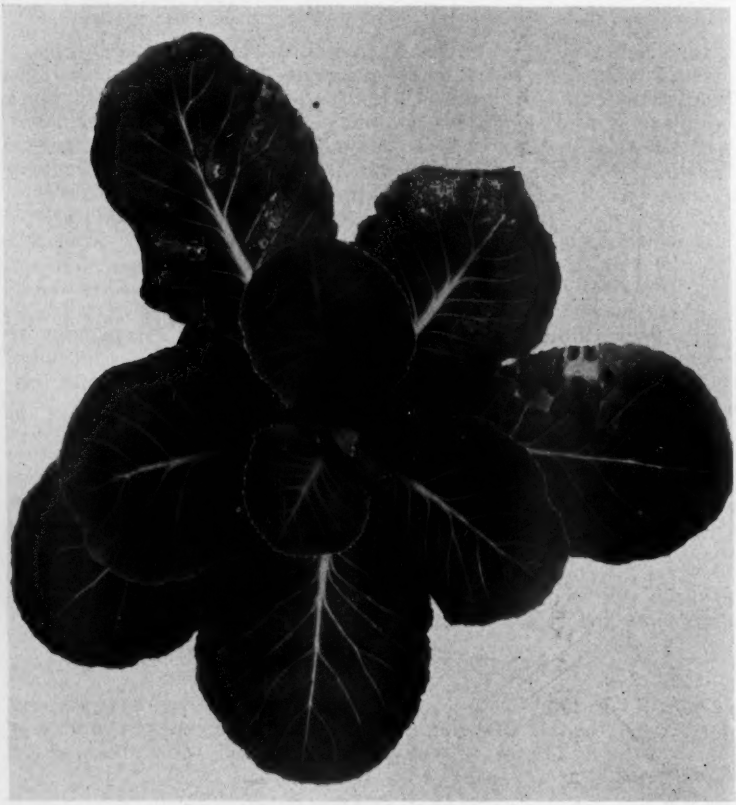

FIGURE 1.—Young cabbage plant infected with the ring necrosis virus, showing small chlorotic lesions on younger leaves and necrotic ring development on the more mature leaves.

Brassica oleracea var. *gemmifera* Zenker (brussels sprouts, var. Long Island Improved).
Brassica oleracea var. *viridis* L. (kale, var. Dwarf Green Curled).
Brassica campestris var. *napobrassica* DC. (rutabaga, var. American Purple Top).
Brassica napus L. (rape, var. Dwarf Essex).
Brassica rapa L. (turnip, var. Purple Top White Globe).
Brassica juncea (L.) Coss. (leaf mustard, var. Tender Green).
Brassica pekinensis (Lour.) Rupr. (Chinese cabbage, var Chihli).
Brassica hirta Moench (*B. alba* (L.) Boiss, white mustard).
Brassica nigra (L.) Koch (black mustard).
Cheiranthus allionii Bailey (Siberian wallflower).

Mathiola incana var. *annua* (L.) Voss (annual stock, var. Dwarf Ten Weeks).
Hesperis matronalis L. (dames rocket).
Raphanus sativus L. (radish, var. French Breakfast).
Berteroa incana DC. (hoary alyssum).
Capsella bursa-pastoris (L.) Medic. (shepherds-purse).
Lepidium virginicum L. (wild peppergrass).
Lepidium sativum L. (garden cress).
Thlaspi arvense L. (pennycress).
Neslia paniculata (L.) Desv. (ballmustard).
Sisymbrium officinale (L.) Scop. (hedgemustard).
Sisymbrium altissimum L. (tumblemustard).

The symptoms on some of these hosts will be described.

Cabbage

No symptoms appear on inoculated leaves of cabbage (*Brassica oleracea* var. *capitata*), but 17 to 21 days after inoculation small con-

Figure 2.—A cabbage leaf affected with ring necrosis. Note various stages of transition from the chlorotic spot through the development of a necrotic center and marginal ring to the final "bull's-eye" lesion.

spicuous yellow lesions appear in the parenchyma of the fourth or fifth unfolded leaf, at the distal portion from the growing tip (fig. 1). The more intense chlorosis at the outer margin of the lesions gives the appearance of a halo. Since the lesions are usually numerous they

may impart collectively the effect of mottling, but there is neither true interveinal mosaic nor systemic vein clearing. The lesions gradually increase in size and number over the entire leaf blade.

FIGURE 3.—Outer leaf of diseased mature cabbage plant showing necrotic rings, vein necrosis, and chlorotic bleaching.

As the leaves become older necrosis appears as spots in the center of the lesion and a little later as concentric rings at the outer margin, giving in the final stage a "bull's-eye" spot in which the dead tissue is brown or blue black (fig. 2). As the disease progresses the tissue

between the initial lesions bleaches and finally becomes dry and brittle (fig. 3). At this stage the leaves drop prematurely, but leaf drop is generally less extensive than in cabbage mosaic (*2*). Affected leaves occasionally develop asymmetrically. Curling or crinkling of the lamina of the leaf is accentuated only in the later

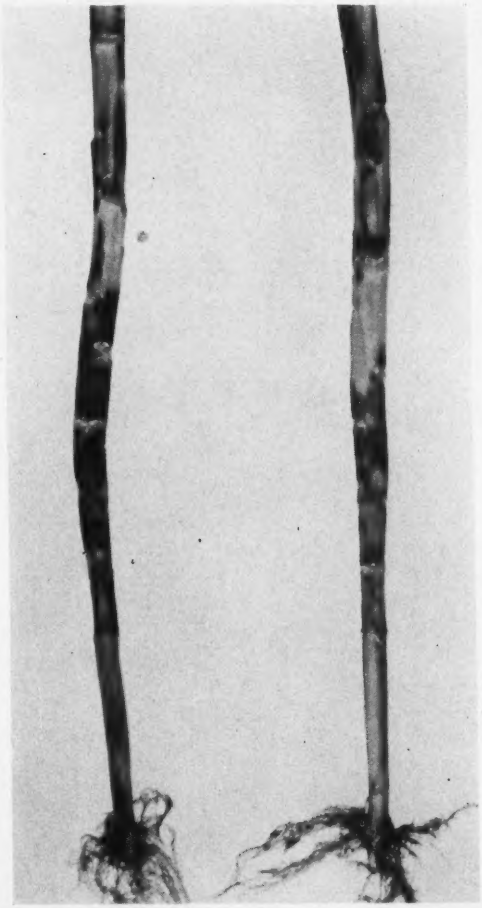

FIGURE 4.—Stems of young cabbage plants infected with ring necrosis virus showing irregular blue-black lesions on the internodes.

stages of the disease. Necrosis may occur on or along the veins after the necrotic rings in the leaf parenchyma have formed.

Neither Smith (*4*) nor Tompkins et al. (*9*) mentioned stem necrosis, which in the present disease commonly occurs in the form of irregular, dark blue to black, slightly sunken areas. The lesions may be small and linear or they may extend irregularly for some distance, usually not involving the leaf scars (fig. 4).

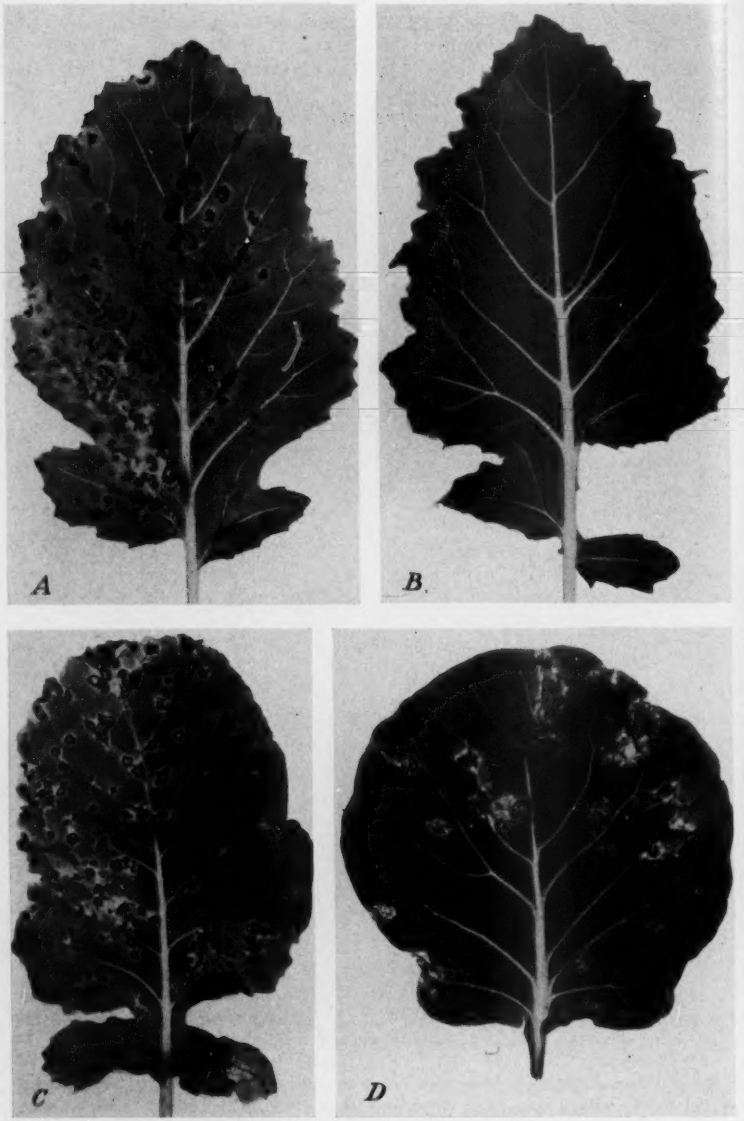

FIGURE 5.—Leaf symptoms of ring necrosis on subspecies of *Brassica oleracea*. *A*, *B*, Kohlrabi: *A*, Diseased; *B*, uninoculated control; *C*, Sprouting broccoli; *D*, brussels sprouts.

Field symptoms vary considerably. Plant growth may be stunted without any sign of chlorosis or necrosis except on the outer head leaves. Outer leaves show varied effects, including chlorosis, unilateral distortion, necrotic rings in interveinal tissue, and linear necrotic lesions on or along the veins. Bleaching of older infected leaves and stem necrosis may occur. Leaf drop is not common as in mosaic, and the internal necrosis of cabbage heads noted in the latter disease (*2*) has not been observed.

Other Members of Brassica oleracea

In kale (*Brassica oleracea* var. *viridis*), brussels sprouts (*B. oleracea* var. *gemmifera*), cauliflower (*B. oleracea* var. *botrytis*), sprouting broccoli (*B. oleracea* var. *botrytis*), and kohlrabi (*B. oleracea* var. *gongylodes*), the first symptoms, as in cabbage, appear as small halolike chlorotic lesions, which develop necrotic rings (fig. 5). Conspicuous linear necrotic lesions appear on veins, petioles, and stems.

Chinese Cabbage

Chinese cabbage (*Brassica pekinensis*) is very susceptible and exhibits somewhat erratic systemic symptoms. The first evidence

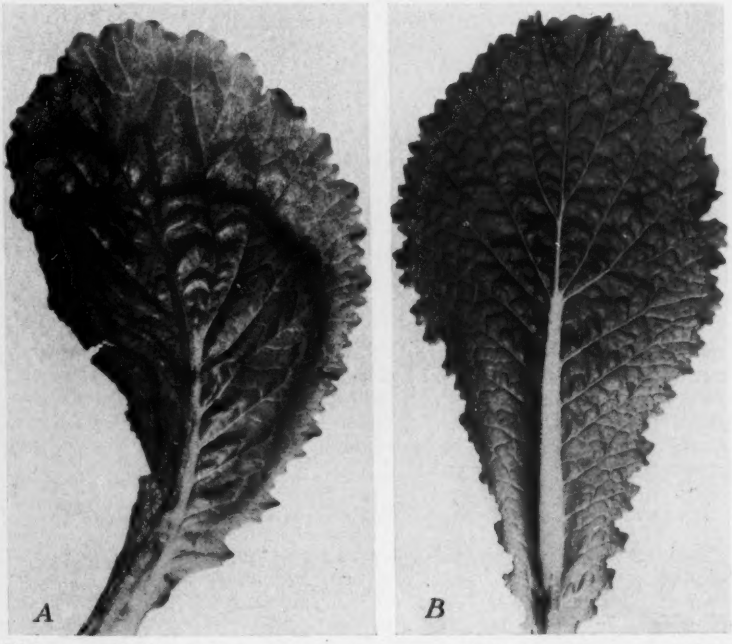

Figure 6.—Leaf symptoms produced by systemic infection of Chinese cabbage with ring necrosis virus: *A*, Leaf distortion, vein necrosis, and necrotic streaking of petiole; *B*, uninoculated control.

of infection is the appearance of numerous dark specks in the interveinal tissue with slight necrotic streaking on the veins. The lesions may be scattered irregularly but are often confined to one side of the

leaf blade; later when necrotic vein streaking increases and involves the petiole, the symptoms (fig. 6) resemble somewhat those of systemic infection by the black-rot organism, *Bacterium campestre* (Pammel) E. F. Smith (*Phytomonas campestris* (Pammel) Bergey et al.). Well-marked progressive necrotic streaking, severe malformation, and stunting are evident in older infected leaves. The tissues gradually become dry and brittle, and the leaves die prematurely. The virus is occasionally lethal. In contrast to these disease manifestations, Tompkins et al. (*9*) described only chlorotic rings, some of which became necrotic, on Chinese cabbage infected with black ring.

White Mustard

Plants of white mustard (*Brassica hirta*) infected with the ring necrosis virus show slight chlorotic lesions on systemically infected leaves, but before ring symptoms fully develop the plants usually die. Tompkins et al. (*9*) report that black ring disease on this host produces chlorotic rings confined to inoculated leaves with vein clearing on new inner leaves.

Annual Stock

In systemically infected leaves of annual stock (*Mathiola incana* var. *annua*), chlorotic mottling and vein banding develop, followed by bleaching and necrosis. The plants are usually stunted. The flowers show breaking in the form of light flecks or streaks. In black ring on this host (*9*), mottling and bleaching of the leaves and breaking of flowers occur.

Dames Rocket and Siberian Wallflower

In dames rocket (*Hesperis matronalis*) and Siberian wallflower (*Cheiranthus allionii*), systemic infection results in stunting, conspicuous mottling, and some malformation (fig. 7). The symptoms on these hosts are not unlike those caused by the cabbage mosaic virus (*2*).

Rape, Turnip, Rutabaga, and Radish

In rape (*Brassica napus*), turnip (*B. rapa*), rutabaga (*B. campestris* var. *napobrassica*), and radish (*Raphanus sativus*), the early lesions on systemically infected leaves are chlorotic at first, and, as in cabbage, necrotic rings develop later. Various degrees of leaf distortion and stunting occur. It is to be noted that the black ring virus did not infect radish (*9*).

Shepherds-Purse, Garden Cress, Pennycress, and Hoary Alyssum

Shepherds-purse (*Capsella bursa-pastoris*), garden cress (*Lepidium sativum*), pennycress (*Thlaspi arvense*), and hoary alyssum (*Berteroa incana*) exhibit slight vein clearing with dwarfing of new growth and chlorosis. In 10 to 15 days after inoculation, early symptoms on older leaves appear as chlorosis of the interveinal areas with a downward curling of the leaf and slight mottling followed by necrosis and defoliation. The younger leaves are malformed and curled, giving the appearance of rosette disease (fig. 8, *A*).

NONCRUCIFEROUS HOSTS

Tompkins et al. (*9*) secured systemic infection with the black ring virus in lambsquarters (*Chenopodium album* L.), sowbane (*C. murale* L.), and spinach (*Spinacia oleracea* L.), although they failed to recover the virus from infected plants. No infection was obtained in sugar beet (*Beta vulgaris* L.) nor Swiss chard (*B. vulgaris* var. *cicla* L.). The writers secured infection in sugar beet, mangel, Swiss chard, and

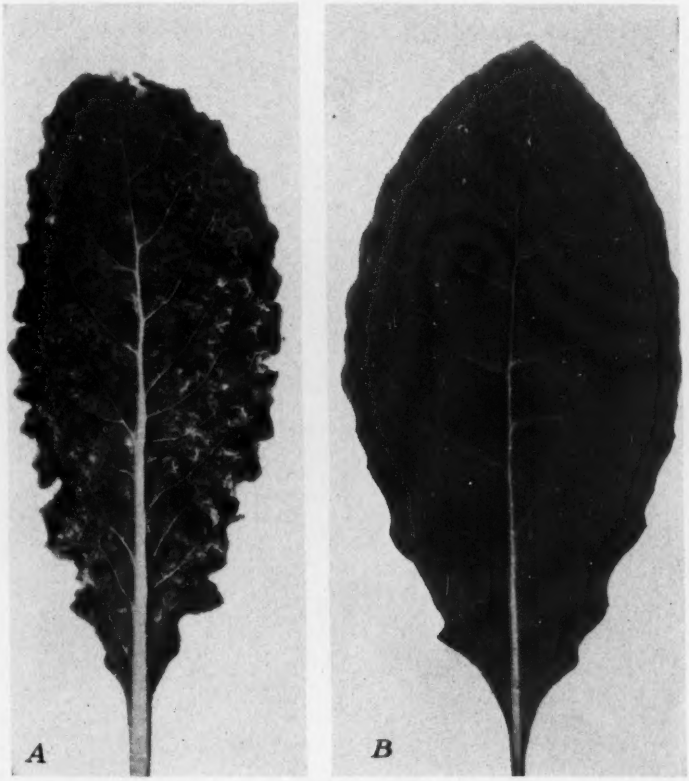

FIGURE 7.—*A*, Leaf from inoculated dames rocket plant infected with the ring necrosis virus, showing conspicuous mottling; *B*, uninoculated control.

spinach with the ring necrosis virus. On the first three of these, numerous small dark local spots appeared at the distal portion of the systemically infected leaf without mottling or vein clearing. The spots increased in size and number, and the entire leaf gradually became dry and brittle. Infected spinach leaves (var. Bloomsdale) showed vein clearing and a conspicuous progressively chlorotic mottling. The growing point was stunted and the plant formed a

pathological rosette, while the leaves became distorted and died prematurely (fig. 8, *C*). No infection occurred on lambsquarters.

Most crucifer viruses are infectious to various members of *Nicotiana*. Smith (*4*) reported that the ring spot virus caused necrotic lesions on inoculated leaves of tobacco (*N. tabacum* L.) in 7 to 10 days. On *N. langsdorffii* Schrank, local lesions and systemic mottling occurred. On *N. glutinosa* L., large local necrotic lesions appeared on inoculated

FIGURE 8.—*A*, *B*, Shepherds-purse: *A*, Symptoms of systemic infection by ring necrosis virus, showing chlorosis, leaf curl, and dwarfing; *B*, healthy control. *C*, *D*, Spinach: *C*, Virus-infected plant showing systemic chlorotic mottling and stunting of terminal bud leaves; *D*, healthy control.

leaves, and a mottling of light-green and yellow specks appeared on younger leaves. This disease was commonly fatal to young plants. Rather similar reactions of these three species to the black ring virus are reported (*9*).

In the case of the ring necrosis virus, necrotic lesions appeared in 3 to 4 days on tobacco (var. Connecticut Havana No. 38). They enlarged rapidly up to 3 mm. or more in diameter, the necrotic tissue forming concentric rings around a reddish center with a darker band

at the margin (fig. 9). No systemic infection occurred. In *Nicotiana glutinosa* no local lesions occurred. The early systemic symptoms are small diffuse chlorotic lesions that become more conspicuous as slight

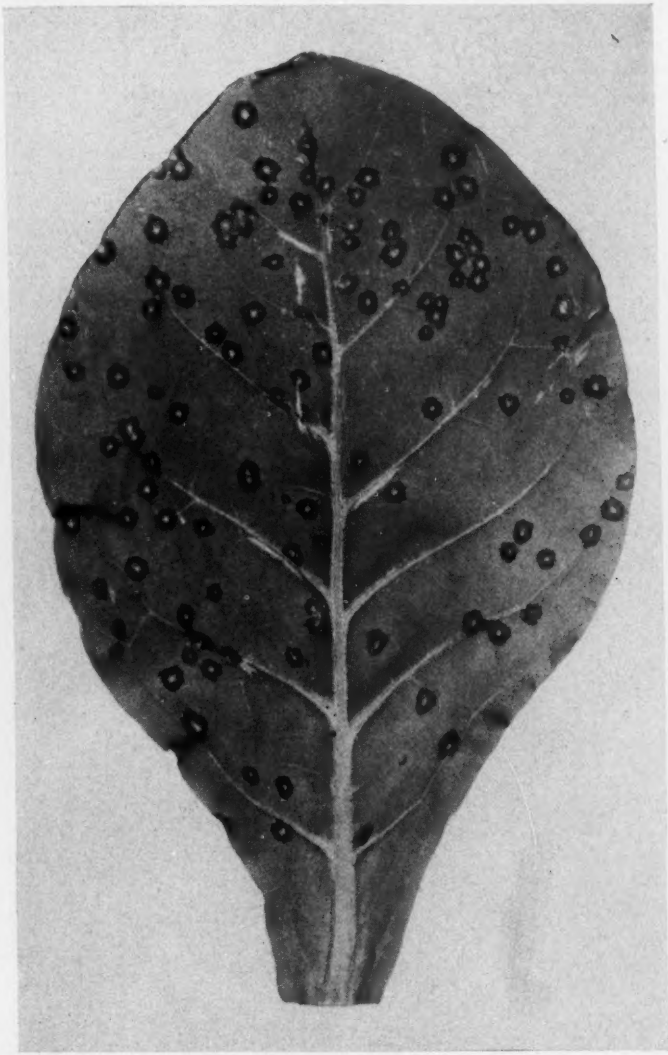

FIGURE 9.—Necrotic local lesions produced on leaf of *Nicotiana tabacum* by ring necrosis virus.

necrosis develops at the margins. Necrosis gradually involves the entire leaf, but the virus is not fatal to the plant (fig. 10, *A*). In *N. langsdorffii* as well as in *N. rustica* L. and *N. repanda* Lehm., there

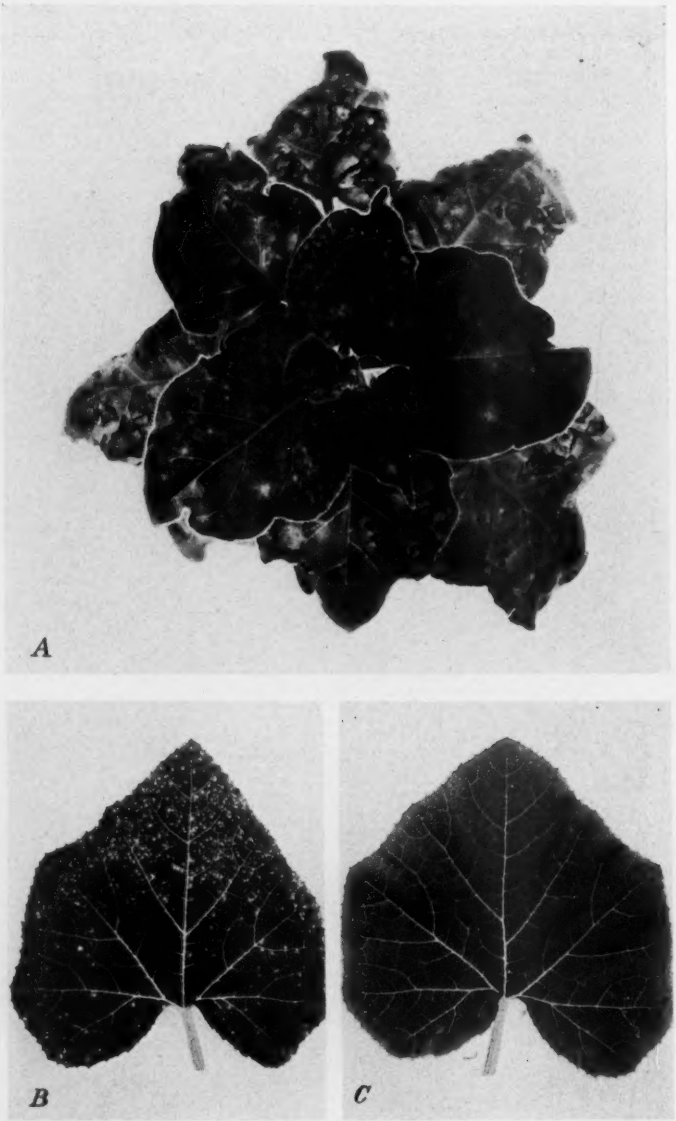

FIGURE 10.—*A*, Systemic infection of *Nicotiana glutinosa*, showing conspicuous chlorotic lesions on younger leaves and necrotic areas on older infected leaves. *B*, Numerous small scattered chlorotic lesions on systemically infected leaf of cucumber; no symptoms appear on the inoculated leaves. *C*, Leaf from healthy cucumber plant (uninoculated control).

are likewise no local lesions. The systemic symptoms, like those of the cabbage mosaic virus (*2*), are chlorosis and pronounced mottling (fig. 11).

In petunia (× *Petunia hybrida* Vilm.) the black ring virus caused chlorotic rings, curling and puckering of leaves, and stunting. Infection of this plant (var. Summer Pink) with ring necrosis virus is characterized by slight vein clearing and small irregular scattered chlorotic lesions with little or no necrosis. Bleaching and dropping of leaves follow, and there is considerable stunting. Sectorial breaking

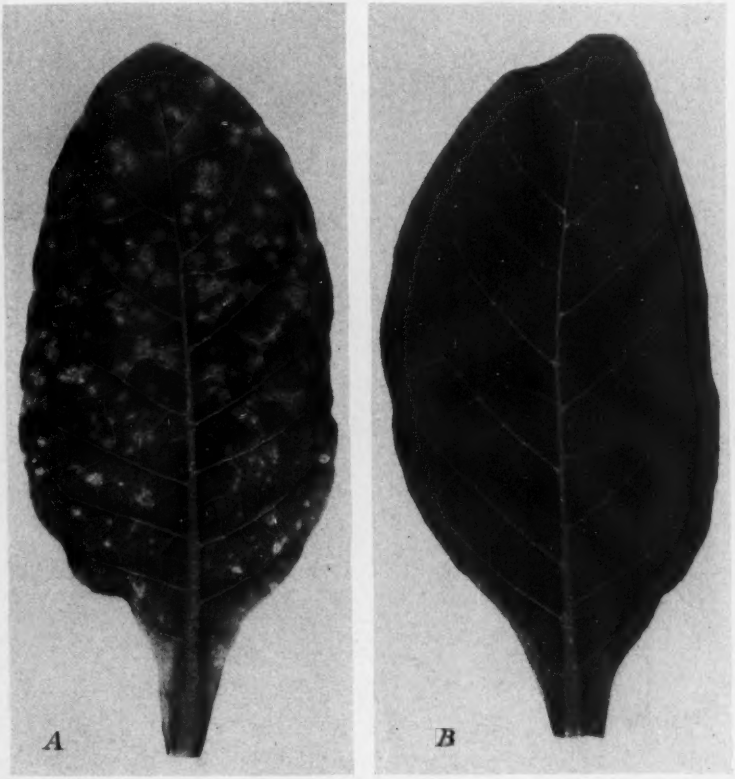

FIGURE 11.—Symptoms produced on leaves of *Nicotiana langsdorffii* by systemic infection with ring necrosis virus: *A*, Systemically infected leaf showing conspicuous, irregular chlorotic lesions; *B*, leaf from uninoculated contral.

in the form of streaks results in malformed and undersized flowers (fig. 12, *A* and *C*).

Zinnia (*Zinnia elegans* Jacq., var. Fantasy Yellow) when infected shows vein clearing and slight mottling followed by leaf twisting and severe stunting. In calendula (*Calendula officinalis* L., var. Ball's Orange) the first symptoms appear as vein clearing and are followed by chlorosis and stunting.

The black ring virus did not infect cucumber (*Cucumis sativus* L.). When the ring necrosis virus was inoculated in the Chicago Pickling

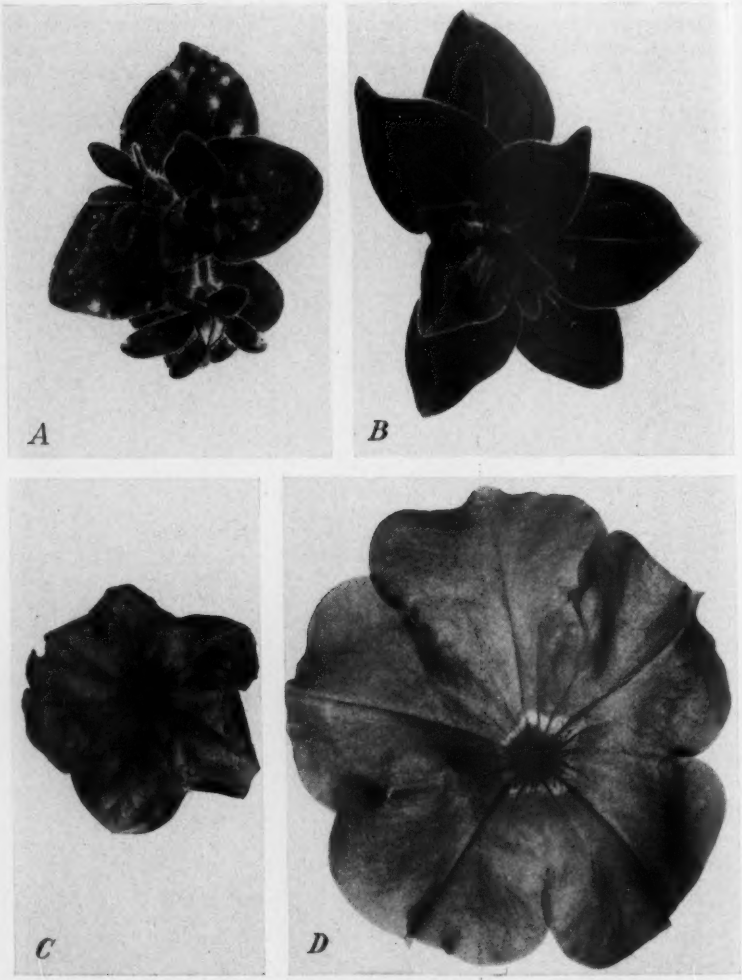

FIGURE 12.—Symptoms produced by ring necrosis virus on petunia: *A*, Vein clearing and scattered chlorotic lesions of leaves on normal green background; *B*, uninoculated control; *C*, sectorial breaking of flower, accompanied by distortion and stunting; *D*, uninoculated control.

variety systemic symptoms appeared as numerous small chlorotic lesions (fig. 10, *B*).

Infection was not obtained on the following species in attempts to inoculate them by the abrasive method or by the transfer of aphids known to be vectors of the ring necrosis virus: Lambsquarters (*Chenopodium album* L.); broadbean (*Vicia faba* L.); nasturtium (*Tropaeolum majus* L.), var. Golden Gleam; pansy (*Viola tricolor* L.), var. Black King; celery (*Apium graveolens* L.), var. Golden Self-Blanching; tomato (*Lycopersicon esculentum* Mill.), var. Globe; currant tomato (*L. pimpinellifolium* (Jusl.) Mill.); potato (*Solanum tuberosum* L.), var. Irish Cobbler and Rural New Yorker; nightshade (*S. nigrum* L.); eggplant (*S. melongena* L.), var. Black Beauty; *Nicotiana sanderae* Bailey; *N. longiflora* Cav.; *N. nudicaulis* S. Wats.; jimsonweed (*Datura stramonium* L.); snapdragon (*Antirrhinum majus* L.), var. (rust-resistant strain); muskmelon (*Cucumis melo* L.), var. Milwaukee Market; watermelon (*Citrullus vulgaris* Schrad.), var. Stone Mountain; dandelion (*Taraxacum officinale* Weber); China-aster (*Callistephus chinensis* Nees), var. Giant Blue (wilt-resistant strain); French marigold (*Tagetes patula* L.), var. Harmony; and florists cineraria (*Senecio cruentus* (L'Hér.) DC.), var. Multiflora Nana. Subsequent reinoculations to young cabbage and tobacco with the extracted juice from the foregoing inoculated plants failed to cause infection.

TRANSMISSION OF THE VIRUS

The strain of the ring necrosis virus used in the experimental studies on insect transmission was obtained from systemically infected mature cabbage grown at Madison, Wis. After the establishment of the virus on healthy cabbage seedlings by mechanical inoculation, a constant source of fresh inoculum was maintained in the greenhouse on this host. The virus remained virtually unchanged through successive transfers. All inoculations were conducted in a greenhouse where the temperature usually ranged from 22° to 25° C. and where weekly fumigation was practiced for the control of insects. The artificial inoculations of test plants were regularly made by dusting the leaves with powdered carborundum and lightly rubbing with a small piece of absorbent cotton soaked with juice extracted from diseased cabbage plants.

Studies on insect transmission of this virus involved the use of the green peach aphid (*Myzus persicae* (Sulz.)) and the cabbage aphid (*Brevicoryne brassicae* (L.)). The methods of culture and transfer of the aphids were essentially the same as those described in a recent paper (*2*). Healthy cabbage seedlings infested with nonviruliferous aphids and uninoculated plants free of aphids served as controls. Reinoculations from all test plants for the recovery of the virus were made to cabbage seedlings to determine the presence of the ring necrosis virus. It was determined by repeated trials that both species of aphid transmit the ring necrosis virus readily.

PROPERTIES OF THE VIRUS

Undiluted juice expressed from recently infected cabbage was used for the study of the properties of the ring necrosis virus. Young cabbage plants were used as test plants. The results are given in table 1.

TABLE 1.—*Properties of ring necrosis virus in vitro as determined by infection on cabbage*

[25 plants used in each test]

Longevity in vitro				Thermal inactivation				Tolerance to dilution			
Period aged at 20°–22° C. (hours)	Plants infected in trial No.—			Temperature (10-minute period)	Plants infected in trial No.—			Dilution	Plants infected in trial No.—		
	1	2	3		1	2	3		1	2	3
	No.	*No.*	*No.*	*°C.*	*No.*	*No.*	*No.*		*No.*	*No.*	*No.*
0	25	25	25	40	25	25	25	0	25	23	24
6	25	25	25	43	24	22	23	1:25	22	23	24
12	24	23	22	45	22	21	20	1:50	16	15	14
24	18	16	20	48	14	15	13	1:100	6	5	7
36	3	2	4	50	0	0	0	1:500	2	2	2
48	0	0	0	52	0	0	0	1:600	0	0	0
60	0	0	0	54	0	0	0	1:700	0	0	0

In table 2 the inactivation points recorded in the literature for other crucifer viruses are listed in comparison with those of the ring necrosis virus. Insofar as these physical properties are concerned, the latter resembles most closely the cabbage black ring virus and the cabbage mosaic virus, but differs from them very definitely in having lower thermal and dilution inactivation points. It has many points of difference in host range and symptoms from all others except the cabbage black ring virus. As already stated, in symptoms produced on cabbage it resembles the black ring and ring spot viruses. The last of these is not described adequately enough by Smith (*4*) to permit comparison, because no physical properties were recorded and the host range and symptoms were studied in only a limited way. The chief distinctions between the ring necrosis virus and the black ring virus, aside from the differences in physical properties just mentioned, are infectivity of radish, sugar beet, and chard by the former, the difference in optimal temperatures for disease development, and differences in symptoms on various common hosts already mentioned.

TABLE 2.—*Inactivation points of ring necrosis virus for longevity in vitro and for resistance to high temperatures and dilutions, compared with those recorded for other crucifer viruses*

Virus	Authority	Point at which inactivation occurred after indicated treatment		
		Period aged in vitro	Temperature (10-minute period)	Dilution
		Hours	*°C.*	
Ring necrosis	Larson and Walker	48	50	1:600
Turnip mosaic	Hoggan and Johnson (*1*)	72	54	1:100,000
Cauliflower mosaic	Tompkins (*5*)	360	75	1:2,000
Chinese cabbage mosaic	Tompkins and Thomas (*10*)	96	75	1:6,000
Turnip mosaic	Tompkins (*6*)	48	63	1:4,000
Cabbage black ring	Tompkins et al. (*9*)	48	57	1:1,000
Mild stock mosaic	Tompkins (*7*)	144	60	1:5,000
Severe stock mosaic	do	192	60	1:4,000
Radish mosaic	Tompkins (*8*)	384	68	1:15,000
Cabbage mosaic	Larson and Walker (*2*)	72	55	1:2,000

SUMMARY

Ring necrosis is a virus disease of cabbage, which is here described in comparison with other crucifer virus diseases.

The virus infects all crucifers tested, including some 25 species and subspecies or botanical varieties, and certain other noncruciferous hosts, including sugar beet, chard, spinach, tobacco, *Nicotiana glutinosa*, *N. langsdorffi* *N. rustica*, *N. repanda*, petunia, zinnia, and calendula.

The virus is transmitted readily by mechanical inoculation and by the green peach and cabbage aphids.

The physical properties investigated are distinct from those already reported for other crucifer viruses.

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MERCURIC CHLORIDE AS A PRESERVATIVE OF CYANOGENETIC PLANTS FOR CHEMICAL ANALYSIS[1]

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INTRODUCTION

In a previous publication[2] it was shown that cyanogenetic plants could be preserved for chemical analysis by means of mercuric chloride for periods ranging from 3 months for dried plants to 6 months for fresh plants. Attention was called to the fact that plants so preserved gave higher yields of hydrocyanic acid on analysis than plants not so preserved, indicating that the customary methods of analysis lead to low results in most cases with considerable error in a number of instances.

The study was continued to determine how long samples of cyanogenetic plants might be preserved with different quantities of mercuric chloride without undergoing appreciable loss of hydrocyanic acid. Since mercuric chloride exerts an inhibiting effect on the enzyme, the study was designed also to determine the length of time the enzyme requires to liberate the maximum obtainable amount of hydrocyanic acid with the different concentrations of mercuric chloride used.

EXPERIMENTAL PROCEDURE

The general procedure was the same as that outlined in the earlier publication.[3]

Fourteen varieties of sorghum grown at the United States Bureau of Plant Industry experimental farm at Arlington, Va., were placed at the disposal of the authors through the courtesy of John H. Martin of that Bureau. During the years 1937 and 1938, 159 collections of material from these varieties were made. The tissues were comminuted either by slicing young plants finely or by mincing older plants in a food chopper. The product was immediately mixed thoroughly and samples, usually 50 gm., of the mixed mass were weighed for the various treatments as rapidly as possible to minimize the loss of hydrocyanic acid which occurs when ground green plant is exposed to the air. In order to obtain uniformity at the beginning of the experiment, in a series of samples that were to be compared, all the samples were covered with water at the same time. The hydrocyanic acid content in one sample was then determined in water by immediate distillation without maceration and in another after maceration for 24 hours. The remainder of the samples were weighed into pint fruit jars, which were then filled with water containing, in solution, 1 to 5 percent of mercuric chloride, calculated on weight of plant. The preserved samples were analyzed at intervals of several weeks to 2 years. The number of samples preserved from each collection depended on the

[1] Received for publication October 17, 1940.

[2] BRIESE, REINHOLD R., and COUCH, JAMES F. PRESERVATION OF CYANOGENETIC PLANTS FOR CHEMICAL ANALYSIS. Jour. Agr. Res. 57: 81-107, illus. 1938.

[3] BRIESE, REINHOLD R., and COUCH, JAMES F. See footnote 2.

quantity of material and time available and on the object in view. A moisture determination was made on each collection of plant. Care was exercised to see that the volume of water used in the preservations was uniform and not large. It has been found that diluting the preservative with water during maceration diminishes its preserving power. When mercuric chloride was added in proportion to the increased volume of water added, the preserving power was maintained, but a longer time was required for complete cyanogenesis in the sample.

Samples of two species of wild cherry (*Prunus melanocarpa* and *P. serotina*) and one species of arrowgrass (*Triglochin maritima*) were also obtained in 1937 and 1938 and preserved in 1 to 5 percent of mercuric chloride.

The samples of *Prunus melanocarpa* and of *Triglochin maritima* were collected, ground, and preserved by E. A. Moran of the Bureau of Animal Industry at the experiment station at Salina, Utah. The material was preserved immediately after collection and no moisture determinations were made. The samples were then shipped to the laboratory in Washington, D. C., where the analyses were made. Under these conditions it was not possible to carry out the analyses in water by immediate distillation or after 24-hour maceration.

The *Prunus serotina* was collected in Arlington, Va., immediately before use. The leaves were ground in a food chopper, mixed, and rapidly weighed, usually 50-gm. samples being taken. At this point the series of samples was quickly flooded with water to assure the greatest possible uniformity in the series, as in the sorghums. The hydrocyanic acid content of one sample was then determined in water by immediate distillation without maceration and in another after maceration for 24 hours. The remainder of the samples were preserved in 1 to 5 percent of mercuric chloride calculated on weight of plant and analyzed at suitable intervals. A moisture determination was made on each collection of *P. serotina*.

SOURCES OF ERROR

The greatest difficulty in preservation was encountered with young plants, owing to the fact that cyanogenesis is more rapid in young tissue, and loss of hydrocyanic acid while the samples are being prepared for preservation is unavoidable. This loss would be less in routine analysis because fewer samples would be used than in the present experiment and less time, therefore, would elapse between mincing or slicing of the plants and immersion in the preservative. This fact accounts in part for the 9 out of a total of 145 analyses in which the values for the unpreserved samples were higher than or equal to those for samples preserved in mercuric chloride. In the early part of the season of 1937, before the main sources of error were recognized, the samples for analysis without preservation were weighed first and sometimes immersed in water before the remainder of the samples were weighed for preservation. In order that the sample for immediate analysis might be distilled by noon and the sample for 24-hour maceration might be ready for distillation the next day early enough to be finished by noon, these two samples were immersed in water as soon as they had both been weighed. Meanwhile, weighing of the series was suspended for a few minutes.

Distillation was begun immediately on the first sample. The samples for preservation were then rapidly weighed, but the short time that had elapsed was no doubt sufficient to allow a greater loss of hydrocyanic acid than in the case of the two samples not preserved. This source of error was eliminated as soon as the 4-week preserved samples were analyzed and the error discovered. Even with this advantage, more than 93 percent of the unpreserved samples gave lower results than were obtained after preservation. In some cases the former were 20 to 30 percent lower than the latter.

In comparing the samples for the efficiency of the preservative in different strengths and for different periods of time, 3 percent was allowed for experimental error, this allowance being based on previous findings. Differences greater than 3 percent were considered as significant.

EFFICIENCY OF 1 PERCENT OF MERCURIC CHLORIDE

In the experiments involving 1 percent of mercuric chloride, the results obtained with sorghums are presented in tables 1 and 2, and those with wild cherry and arrowgrass in table 3.

TABLE 1.—*Hydrocyanic acid obtained from green sorghum in 1937 after maceration in water only and in water containing 1 percent of mercuric chloride calculated on weight of plant*

Collection No.	Variety	Height of plant	Moisture content	Hydrocyanic acid per 100 gm. of dry plant obtained after —							
				No maceration	Maceration for 24 hours in water	Maceration with 1 percent of mercuric chloride in solution for the designated number of weeks					
						4	12	26	52	78	104
		Inches	*Percent*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
99	Ajax	60–72	68.62		16	16					
17	Chiltex	8–10	80.73	41		46					
15	Dwarf yellow milo	12–14	81.11	21		43					
16	do	10–13	83.75	81		127	132				
26	do	30	79.08		53	67	75	69	68		76
43	do	8–10	87.14			221	215	218	215		206
22	Hegari	18	85.42	62	99	108					
30	do	30–36	79.63	25	41	47			44		
57	do	36–48	76.86	15		35		35			
60	do	20–22	80.40	140	225	238					
62	do	36	75.54	129	149	244		250	242		226
72	do	60–72	76.93	81	122	189	182	188	182	191	174
81	do	60	73.14	45	107	124		135			
83	do	6–12	88.91	243	330	341	336	344			
84	do	60–72	70.46	67	133	146					
89	do	60–72	74.75	56	126	147					
96	do	60–72	70.82	33	83	93		88			66
102	do	60–72	70.79	24	55	63		63			
103	do	60–72	74.19	33	55	68		77			
104	do	72–84	76.23	21	42	50	47	48			
105	do	72	73.05	17	28	31					
107	do	72	72.22	15		45		44			
112	do	72–84	62.76	10	21	28					
116	do	72–84	70.70	13	23	29		24			
19	Kansas Orange sorgo	16–18	86.71	55	92	111		110	110		
20	do	6– 8	83.03	97	119	122					
42	do	6– 8	85.11			234	263		263		
32	Sharon kafir	3– 6	83.49	92	104	98	111	107	104		101
46	do	10–12	85.58	101	126	139					
49	do	10–12	86.23	53	104	116			112		
52	do	14–16	87.14	82	96	138	140				
55	do	20–22	84.03	57	76	96					
59	do	30–36	86.42	119	174	182					
63	do	36		32	57	65					

TABLE 1.—*Hydrocyanic acid obtained from green sorghum in 1937 after maceration in water only and in water containing 1 percent of mercuric chloride calculated on weight of plant*—Continued

Collection No.	Variety	Height of plant	Moisture content	Hydrocyanic acid per 100 gm. of dry plant obtained after—							
				No maceration	Maceration for 24 hours in water	Maceration with 1 percent of mercuric chloride in solution for the designated number of weeks					
						4	12	26	52	78	104
		Inches	*Percent*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
68	Sharon kafir	36	77.12	34	69	79	75	82	----	----	----
74	do	60	82.23	32	61	70	65	63	----	----	----
77	do	60–72	75.55	29	58	73	----	68	----	----	----
87	do	60–72	74.15	13	24	31	28	27	----	----	----
94	do	60–72	74.52	11	9	20	----	15	----	----	----
101	do	72	76.10	12	20	21	----	18	----	----	----
111	do	72	74.78	8	10	11	----	----	----	----	----
115	do	72	74.06	12	13	16	----	----	----	----	----
120	do	72	72.95	8	8	15	----	----	----	----	----
14	Spur feterita	3–6	82.81	278	297	277	----	----	----	----	----
27	do	3–5	82.95	263	303	271	----	----	----	----	----
34	do	24–30	82.23	50	89	96	96	----	----	----	----
44	do	3–6	85.87	----	----	290	----	----	292	----	----
45	do	8	85.45	213	253	265	----	----	275	----	----
47	do	24–30	78.46	24	46	52	----	----	47	----	----
48	do	8–10	86.15	183	190	233	226	240	238	----	----
50	do	30–36	76.27	22	41	47	----	----	48	----	----
51	do	14–16	87.29	205	241	260	----	----	----	----	----
53	do	20–24	80.72	127	176	214	215	----	----	----	----
54	do	20–24	86.80	128	180	188	203	----	----	----	----
58	do	36–48	85.20	76	91	102	----	91	----	----	----
64	do	48–60	76.71	114	162	202	----	204	----	----	180
66	do [1]	6–12	85.69	191	220	270	----	270	264	----	256
67	do [1]	6–12	83.18	137	193	228	----	----	----	----	----
69	do	72	75.63	82	154	172	167	----	----	----	----
73	do	72	73.71	68	121	163	151	151	----	125	----
76	do	72–84	73.17	36	73	81	----	77	----	----	----
78	do	12–20	85.37	121	174	174	----	178	----	----	----
80	do	72–84	72.19	43	86	96	100	96	----	----	----
82	do [1]	12	87.78	237	269	301	303	318	----	----	----
86	do	84–96	72.61	36	76	83	84	83	----	----	----
88	do	84–96	66.24	21	43	49	----	46	----	----	----
93	do	84–96	71.36	31	36	41	41	37	----	----	----
100	do	84–96	70.25	10	16	16	----	16	----	----	----
106	do	60–72	70.50	20	34	48	----	----	----	----	----
110	do	84–96	64.69	21	38	49	----	50	----	----	----
113	do [1]	12	85.41	164	191	228	235	234	----	226	----
114	do	84–96	70.67	12	14	21	----	19	----	----	----
117	do [1]	6	84.12	227	243	300	----	----	----	----	----
119	do	84	72.22	8	8	21	----	----	----	----	----
23	Sumac sorgo	14	86.61	31	53	66	----	67	61	----	66
24	do	20	88.93	54	72	106	----	106	101	----	104

[1] Second-growth plants; all others first growth.

TABLE 2.—*Hydrocyanic acid obtained from green sorghum in 1938 after maceration in water only, and in water containing 1 percent of mercuric chloride (calculated on weight of plant), except as otherwise indicated in footnotes*

Collection No.	Variety	Height of plant	Moisture content	Hydrocyanic acid per 100 gm. of dry plant obtained after—					
				No maceration	Maceration for 24 hours in water	Maceration with mercuric chloride in solution for the designated number of weeks			
						4	8	26	52
		Inches	*Percent*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
63	Ajax	36	74.38	65	103	117			
92	do	72	72.95	28	57	71	71		
108	do	60–72	72.42	19	29	38	38		
105	Atlas sorgo	96–108	74.50	12	24	28	28		
60	Dwarf Yellow milo	60	77.94	44	68	83	[1] 84		
93	do	84–96	71.51	16	25	40	40		
76	Grohoma	72–84	73.67	28	63	69	72		
91	do	72–84	73.73	23	49	56	69		
106	do [2]	10–12	85.67	96	128	139	138		
124	do [2]	6–10	82.63	70	132	158	138	155	
31	Hegari	6	82.51		236	[1] 288		[1] 325	
33	do	6–8	84.36		324		[1] 266	[1] 337	[1] 329
40	do	8–12	89.47		409		[1] 312	[1] 316	
43	do	18–22	81.24		171		[1] 209	[1] 267	
45	do	30–36	83.16		176		[1] 218		
47	do	30	79.08		93		[1] 135	[1] 157	
48	do	30	67.55		113		[1] 121		
49	do	30	75.02		109		[1] 114		
50	do	30	75.91		95		[1] 134		
51	do	30	70.55	60			[1] 99		
52	do	30	79.02	89		115	[1] 117		
53	do	30	78.28	70		129	[1] 142		
54	do	30	71.34		82		[1] 99		
55	do	30	69.94	43	78		[1] 89		
56	do	30	75.73	46	91		[1] 113		
58	do	36	82.44	134	127	173	[1] 193		
61	do	36	81.96	105		88	[1] 73		
62	do	36	81.55	152		159	[1] 193		
64	do	42–48	77.99	121	157	175	[1] 266		
65	do	36	80.73	148	138	142			
70	do	60	81.68	134	140	180	218		
71	do [2]	6–12	89.98	217	276	289	300		
73	do	48	82.40	77	144	128	151		
74	do	36–48	81.80	77	99	132	145		
79	do	60–72	72.74	78	89	145	154	159	157
87	do	72–84	76.62	68	127	136	141		
95	do [2]	6–10	87.91	280	288	323	367	385	379
97	do	72–84	74.92	59	129	140			
102	do	72–84	78.10	40	70	88	82		
107	do	60–72	83.64	95	193	208	210		
110	do	72–84	74.46	28	58	72	69	66	
112	do [2]	6–10	88.20	214	385	439	{482 / [1] 474}		
113	do	72–84	72.71	15	26	32	{31 / [1] 32}	32	
118	do	72–84	73.25	47	86	99	104	[1] 105	
127	do [2]	6–8	85.55	216	263	329	397		
84	Kansas Orange sorgo	84–96	75.61	46	78	93	101		
98	do	96	75.82	19	54	57	63	66	
123	do [2]	10–12	86.62	37	53	98	98	106	
81	Leoti sorgo	96	72.55	24	56	59	60		
96	do	96	74.60	13	27	35	35		
83	Rex	96	75.84	33	48	65	68		
99	do	96	68.75	13	21	29	26		
78	Sagrain	72–84							
94	do	72–84	75.06	15	42	43	43		
116	do [2]	6–10	87.01	95	140	155	154		
34	Sharon kafir	8–12	85.26		145		[1] 168	[1] 175	[1] 174
46	do	36	83.91		75		[1] 88	[1] 108	
67	do	48–60	78.30	24	43	48	50		
75	do	72	76.51	19	46	41	41		
82	do	72	76.49	23	33	34	38		
90	do [2]	6–12	85.59	79	89	104	106	109	110
103	do	60–72	75.05	16	25	29	29		
115	do [2]	12–14	86.41	35	36	93	97	94	85
125	do	72	72.11	10	14	13	16	22	

[1] 2 percent of mercuric chloride.
[2] Second-growth plants; all others first growth.

TABLE 2.—*Hydrocyanic acid obtained from green sorghum in 1938 after maceration in water only, and in water containing 1 percent of mercuric chloride (calculated on weight of plant) except as otherwise indicated in footnotes*—Continued

Collection No.	Variety	Height of plant	Moisture content	Hydrocyanic acid per 100 gm. of dry plant obtained after—					
				No maceration	Maceration for 24 hours in water	Maceration with mercuric chloride in solution for the designated number of weeks			
						4	8	26	52
		Inches	*Percent*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
57	Spur feterita	36–48	80.16	108	145	163	[1] 171	.....	.....
69	....do	72	74.84	110	166	187	188	198	183
86	....do	72–84	75.22	74	122	139	140	.....	.....
88	....do [2]	6–12	86.83	202	211	260	270	.....	.....
104	....do [2]	6–10	87.44	363	388	463	484	478	485
111	....do [2]	8–12	88.22	262	444	447	436	464	413
119	....do [2]	8–14	81.33	46	164	186	190	198	183
122	....do [2]	8–10	85.13	105	227	256	275	.....	.....
126	....do [2]	10–12	85.03	78	154	174	208	190	.....
128	....do [2]	6–7	84.49	146	155	236	.....	.....	.....
35	Sumac sorgo	8–10	85.16	.....	233	.....	[1] 246	.....	[1] 257
42	....do	12–16	85.65	.....	205	.....	[1] 272	.....	.....
59	....do	60	80.81	83	114	158	.....	.....	.....
66	....do	72–84	79.04	62	85	120	[1] 130	[1] 131	.....
77	....do	84–96	72.56	46	95	114	114	.....	.....
80	....do	84–96	73.10	51	100	107	107	.....	.....
89	....do	84–96	76.71	51	90	108	110	.....	.....
114	....do	96	75.23	30	58	76	78	71	.....
120	....do [2]	6–10	87.21	103	176	229	[1] 84 232	258	.....

[1] 2 percent of mercuric chloride.
[2] Second-growth plants; all others first growth.

TABLE 3.—*Hydrocyanic acid obtained from wild cherry (Prunus melanocarpa and P. serotina) and from arrowgrass (Triglochin maritima) after maceration in water only or in water containing 1 percent of mercuric chloride calculated on weight of plant*

Collection No.	Plant	Moisture content	Hydrocyanic acid per 100 gm. of plant macerated in—							
			Water for 24 hours	Mercuric chloride for the designated number of weeks						
				4	8	13	26	52	78	104
		Percent	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
37–69	*Prunus melanocarpa* [1]	.....	.....	139	136	136	[2] 126	118	115	105
37–69A	....do [1]	.....	.....	126	.....	.....	.....	.....	126	.....
37–79	....do [1]	.....	.....	120	122	121	115	112	.....	.....
5–37	*P. serotina* [3]	73.22	428	.....	.....	.....	[4] 462	426	.....	.....
9–37	....do [3]	65.93	281	.....	244	232	249	.....	.....	.....
11–37	....do [3]	65.14	255	252	.....	256	251	238	.....	.....
33–37	....do [3]	62.64	438	457	.....	.....	460	.....	.....	.....
37–61	*Triglochin maritima* [1]	.....	.....	101	.....	.....	.....	98	89	.....
37–62	....do [1]	.....	.....	122	131	130	129	121	114	104
37–62A	....do [1]	.....	.....	59	.....	.....	59	.....	.....	.....
37–68	....do [1]	.....	.....	12	.....	13	10	10	.....	.....

[1] Calculated to green plant for which moisture determinations are not available.
[2] 30 weeks.
[3] Calculated to moisture-free plant.
[4] 35 weeks.

In the case of the sorghums, for each period of time in both years, a count was made of the samples with upward, stationary, and downward trends in hydrocyanic acid content. The data are given in table 4. In determining the trend in a series of samples, it was necessary to ignore certain obvious errors. For example, if the 8-week sample

was lower than the 4-week one, and the 26-week sample was more than 3 percent higher than the 4-week sample, then the trend of the 26-week sample was upward and therefore the trend of the 8-week sample was considered to be upward also.

TABLE 4.—*Percentage of total number of sorghum samples, after indicated number of weeks in 1-percent mercuric chloride in solution, showing trends in hydrocyanic acid content*

Trend	Percentage of total samples after—							
	8 weeks (1938)	12 weeks (1937)	26 weeks (1937)	26 weeks (1938)	52 weeks (1937)	52 weeks (1938)	78 weeks (1937)	104 weeks (1937)
	Percent	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*	*Percent*
Upward	55	43	15	47	6	0	0	0
No change	39	44	51	29	47	38	33	30
Downward	6	13	34	24	47	62	67	70
	Number	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Total samples	51	23	39	17	17	8	3	10

As mentioned previously, more than 93 percent of the unpreserved samples gave lower results than were obtained after preservation. Table 4 shows that of the samples analyzed in 1938 after 8 weeks of preservation, 94 percent were higher than or equal to the 4-week samples in hydrocyanic acid content, no 12-week samples having been considered. Of those analyzed in 1937 after 12 weeks of preservation, 87 percent were higher than or equal to the 4-week samples, no 8-week samples having been considered. For both years 70 percent of the samples preserved for 26 weeks gave figures higher than or equal to those for 8 or 12 weeks. Of the 26-week group, the samples having a downward trend were 17 in number, 4 of which were near the limit of experimental error, 5 were from 6 to 10 percent low, and 8 were from 10 to 20 percent low.

The time required for complete cyanogenesis, as judged from table 4, varied considerably. For the intervals of 8, 12, 26, and 52 weeks the percentages of samples showing an upward trend were 55, 43, 25, and 4, respectively. Thus with 1 percent of mercuric chloride, 25 percent of the samples required between 8 and 26 weeks for complete cyanogenesis.

In the case of wild cherry and arrowgrass (table 3), with the use of 1 percent of mercuric chloride there was good preservation for 8 to 13 weeks and in most instances for 26 weeks. However, it appears that 1 percent is not sufficient to preserve material of these species when the content of hydrocyanic acid is large.

EFFECT OF HIGHER CONCENTRATIONS OF MERCURIC CHLORIDE

Data obtained after the preservation of samples in concentrations of mercuric chloride of 1 to 5 percent and for periods of 4 to 78 weeks, (except for one instance of 104 weeks) are presented for sorghum in table 5 and for wild cherry and arrowgrass in table 6. The higher concentrations of mercuric chloride appeared to give better preservation in some cases but necessitated a longer period for complete cyanogenesis because of the increased inhibition of the enzyme with increasing concentration of mercuric chloride.

TABLE 5.—*Hydrocyanic acid obtained from sorghums after maceration with various quantities of mercuric chloride in solution for different intervals of time*

Collection No.	Variety	Moisture content	Time of maceration	Hydrocyanic acid per 100 gm. of dry plant obtained after maceration in mercuric chloride solution of the designated strengths in percent [1]				
				1	2	3	4	5
		Percent	*Weeks*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
45–38	Hegari	83.16	8	----	218	168	131	95
79–38	do	72.74	4	145	138	124	128	118
			8	154	----	----	----	----
			12	151	153	153	154	----
			26	159	154	159	159	144
			52	157	159	157	160	157
			78	----	161	162	165	166
95–38	do [2]	87.91	4	323	----	----	----	----
			8	367	342	----	----	----
			26	385	389	----	----	----
			52	379	----	----	----	----
			78	380	----	----	----	----
118–38	do	73.25	4	99	----	----	----	----
			8	104	100	----	----	----
			26	----	105	----	----	----
36–130	do [3]	14.02	4	----	68	69	69	70
			13	----	69	68	68	70
			15	----	69	----	----	----
			17	----	68	68	----	----
			19	----	65	70	69	----
			21	----	63	69	70	70
			30	----	----	68	69	68
			39	----	----	----	----	69
			52	----	----	----	68	69
115–38	Sharon kafir	86.41	4	93	----	----	----	----
			8	97	103	----	----	----
			26	94	110	----	----	----
			52	85	91	----	----	----
104–38	Spur feterita [3]	87.44	4	463	----	----	----	----
			8	484	465	----	----	----
			13	496	500	----	----	----
			26	478	479	----	----	----
			52	485	482	----	----	----
111–38	do [3]	88.22	4	447	----	----	----	----
			8	436	420	----	----	----
			26	464	442	----	----	----
			52	413	----	----	----	----
119–38	do [3]	81.33	4	186	----	----	----	----
			8	190	191	----	----	----
			12	202	----	----	----	----
			26	198	199	----	----	----
			52	183	210	----	----	----

[1] Calculated on weight of plant.
[2] Second growths; all others first growth.
[3] Dried plant.

TABLE 6.—*Hydrocyanic acid obtained from wild cherry (Prunus melanocarpa and P. serotina) and from arrowgrass (Triglochin maritima) after maceration in various quantities of mercuric chloride in solution for different intervals of time*

Collection No.	Plant	Moisture content	Time of preservation	Hydrocyanic acid per 100 gm. of dry plant obtained after maceration in—					
				Water	Mercuric chloride solution of the designated strengths in percent [1]				
					1	2	3	4	5
		Percent	*Weeks*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
38-91	*P. melanocarpa*	74.98	8		825				
			26		833	[2] 805			
			52		754	[2] 794			
38-92	do	77.66	8		895				
			26		888	[2] 905			
			52		811	[2] 863			
38-152	do	63.35	8	251	313	309	301	284	293
			13	239	314	311	306	301	318
			26	201	316	311	327	323	327
			52		291	312	318	306	314
38-173	do	61.11	8	302	347	353		347	338
			26	232	348	373	374	359	363
108-37	*P. serotina*	67.01	8		339	[3] 337			
			13		346	[3] 345			
			26		346	[3] 343			
21-38	do	68.05	4			309	308	308	
			12			313	313	304	
			26		299	315	313	316	304
			52		292	321	325	319	305
			104			319		306	
117-38	do	57.75	4		512	535			
			8		515	546			
			12			555			
			26		505	544	531		543
			52		471	503			
			78			543			
38-95	*Triglochin maritima* [4]		8	136	178	166			
			13	143	174	166	160	147	141
			26	132	171	167	159	158	149
			78		155	160	160	155	150

[1] Calculated on weight of plant.
[2] 1 percent of mercuric chloride added 2 months after beginning of experiment.
[3] 1.5 percent of mercuric chloride.
[4] Calculated to green plant.

For sorghum, tables 2 and 5 show that in 12 of 14 cases the 2-percent 26-week samples were higher than the 2-percent 8-week samples. Therefore, in many cases, for 2-percent mercuric chloride more than 8 weeks are required for complete cyanogenesis in sorghum. Of the 26-week samples in 2-percent mercuric chloride, all were higher than or equal to the 1-percent 8-week samples of the same collections. The three comparable 12- and 13-week samples (table 5) showed that in these three cases cyanogenesis was completed in 3 months. In only one case did a 2-percent 26-week sample show a loss as compared with a shorter period. In only one case did a 2-percent 26-week sample with upward trend show a lower value than the 1-percent 26-week sample with upward trend. It seems safe to conclude that, for sorghum in 2-percent mercuric chloride, cyanogenesis will be complete in 3 or 4 months and that the liberated hydrocyanic acid will be preserved in almost all cases for 6 months.

The increased efficiency of 2 percent as compared with 1 percent of mercuric chloride in the preservation of wild cherry (*Prunus serotina*) is seen by comparing the data in tables 3 and 7. In table 7 no losses, except such as were within the limits of experimental error, were

evident in samples preserved in 2 percent of mercuric chloride for 6 months and in the sample preserved for 1 year. With 1 percent of mercuric chloride (table 3), there was good preservation for 8 to 13 weeks and in most cases for 6 months.

TABLE 7.—*Hydrocyanic acid obtained from fresh wild cherry (Prunus serotina) after maceration in water only or in water containing 2 percent of mercuric chloride calculated on weight of plant*

Collection No.	Hydrocyanic acid per 100 gm. of dry plant after maceration in—						
	Water for—		2-percent mercuric chloride solution for—				
	0 hours	24 hours	4 weeks	8 weeks	13 weeks	26 weeks	52 weeks
	Milligrams	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*	*Milligrams*
20–38	338		378				
21–38	247		309		313	315	321
30–38	648	646	731			734	
32–38	472		553	563			
37–38	356	350	409			410	
117–38	332	454	535	546	555	544	

In the case of green sorghum (second sample in table 5), 2 percent of mercuric chloride preserved as well as 3 percent for the period covered (78 weeks). For dried sorghum (fifth sample in table 5) 3 percent preserved better than 2 percent for a period of more than 20 weeks.

From the data in table 6 it seems safe to conclude that, for wild cherry, in most cases 2 percent of mercuric chloride is as satisfactory a preservative as 3 percent, especially for intervals up to 26 weeks. In the third sample of wild cherry 3 percent of mercuric chloride preserved somewhat better than 2 percent and as well as 4 or 5 percent.

For both sorghum and wild cherry higher concentrations of mercuric chloride than 3 percent do not offer advantages to offset the increased inhibition of cyanogenesis.

In the sample of green *Triglochin maritima* used, however, the highest figures were obtained with 1 percent of mercuric chloride, indicating that the enzyme of this plant is sensitive to the preservative. One percent of mercuric chloride is sufficient to combine with the total hydrocyanic acid found in the sample, since 1 gm. of the salt will combine with 199.2 mg. of the acid. However, many other substances in green plants will combine with mercuric chloride so that an excess of the salt should be employed.

RAPIDITY OF FORMATION OF HYDROCYANIC ACID IN PRESERVED SAMPLES

Since it was known that mercuric chloride inhibits cyanogenesis in plants, it was of interest to determine how rapidly the hydrocyanic acid is formed, particularly during the first 4 weeks of preservation. Data for the rate at 2-hour intervals during the first 24 hours of preservation for wild cherry (*Prunus melanocarpa*), arrowgrass, sorghum, and Johnson grass have already been published,[4] together with a few data for longer intervals up to 13 days. Data obtained at weekly intervals up to 4 weeks, and for longer intervals thereafter,

[4] BRIESE, REINHOLD R., and COUCH, JAMES F. See footnote 2.

with the use of 1 percent of mercuric chloride, are presented in table 8. It was found that in these samples the maximum figure, within experimental error, developed in 3 to 4 weeks with two exceptions, in which the maximum developed at 5 to 13 weeks. Data for 8-week samples are not available, but table 4 shows that for the 1938 sorghums 55 percent of the 8-week samples gave more hydrocyanic acid than the 4-week samples. Table 8 shows that cyanogenesis in the presence of mercuric chloride is rapid for wild cherry but is slow with sorghum. This finding is consistent with other evidence, which indicates that the enzyme in wild cherry is very much more active than in sorghum. The highest yield of hydrocyanic acid that can be obtained by maceration in water is ordinarily obtained after 2 to 3 hours with wild cherry but only after 24 to 48 hours with sorghum.

TABLE 8.—*Rapidity of formation of hydrocyanic acid in green cyanogenetic plants macerated in water containing 1 percent of mercuric chloride calculated on weight of plant*

Collection No.	Plant	Moisture	Hydrocyanic acid per 100 gm. of dry plant obtained after maceration in—							
			Water for 24 hours	1-percent mercuric chloride solution for the designated number of weeks						
				1	2	3	4	13	26	52	104
		Percent	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*	*Mg.*
6-37	*Prunus serotina*	65.60	309	313	312	312	327	-----	-----	[1] 320	303
8-37	do	62.44	[2] 125	121	123	123	123	-----	122	-----	-----
10-37	do	69.08	[2] 303	312	312	312	295	[3] 321	[4] 304	-----	-----
13-37	Spur feterita	85.80	73	77	81	80	82	88	89	82	82
18-37	Sorghum var. Chiltex	83.90	91	95	102	104	101	-----	102	96	-----
21-37	Kansas Orange sorgo	87.72	125	118	126	-----	128	-----	129	121	-----
31-37	Spur feterita	82.25	179	155	157	177	175	175	-----	[5] 180	161

[1] 65 weeks.
[2] Not macerated, the macerated sample being lower.
[3] 5 weeks; same figure for 6 weeks.
[4] 30 weeks.
[5] 78 weeks.

ATTEMPTS TO INCREASE THE RATE OF CYANOGENESIS IN PRESENCE OF MERCURIC CHLORIDE

A number of experiments were made to determine whether a technique might be developed by means of which the time for maximum development of hydrocyanic acid in the presence of mercuric chloride could be shortened. In one experiment 50 gm. of fresh second-growth Spur feterita was heated for 7 hours under a reflux condenser in a mixture of 1,500 cc. of water and 0.5 gm. of mercuric chloride. On the following day the hydrocyanic acid was determined as 139 mg. per 100 gm. calculated to dry plant. The quantity obtained after maceration of this sample for 4 weeks in 1 percent of mercuric chloride was 236 mg. Evidently the refluxing was not effective in causing complete hydrolysis of the glucoside. Other attempts were made in which the quantity of preservative was varied and maceration stopped after 1 to 4 days with and without the addition of an enzyme preparation made from sorghum and known to be active. The results are presented in table 9. A comparison of the quantity of hydrocyanic acid obtained after such treatment with that determined

by 4 to 8 weeks' maceration of the same sample in 1 percent of mercuric chloride shows that, in 13 experiments, the figure obtained by the former method was higher in 1 instance and the same, within experimental error, in 2 instances as with the latter method. In 10 instances results with the latter method were higher, the difference being more than 100 percent in sample No. 64–38 of hegari. In two cases the addition of enzyme made little difference, but with sample No. 63–38 of Ajax, there was a loss in the hydrocyanic acid obtained when enzyme was added.

TABLE 9.—*Comparison of quantity of hydrocyanic acid obtained after adding enzyme to fresh sorghum macerated in mercuric chloride solution, with that obtained from a similar sample macerated for 4 to 8 weeks in water containing 1 percent of mercuric chloride, calculated on weight of plant*

Collection No.	Variety	Enzyme added	Period of maceration	Mercuric chloride added	Hydrocyanic acid per 100 gm. of dry plant obtained after—	
					Treatment	Maceration for 4 to 8 weeks in 1 percent of mercuric chloride
			Days	*Percent*	*Mg.*	*Mg.*
63–38	Ajax	Before maceration	4	0.5	68	117
63–38	do	None	4	.5	90	117
45–38	Hegari	do	1	.5	141	218
58–38	do	do	2	.5	190	193
58–38	do	After maceration	2	.5	208	193
58–38	do	Before maceration	2	.5	197	193
64–38	do	None	2	.5	92	206
27–38	Scarborough broomcorn	do	1	.5	75	153
46–38	Sharon kafir	Before maceration	1	.5	82	108
69–38	Spur feterita	do	3	1.0	177	198
69–38	do	do	7	1.0	183	198
59–38	Sumac sorgo	do	2	.5	124	156
59–38	do	None	2	.5	126	156

STABILITY OF MERCURIC CYANIDE SOLUTIONS

The apparent loss of hydrocyanic acid in the above-cited case of Ajax sorghum raised the question whether mercuric cyanide is stable in the presence of cyanogenetic enzymes. It is known that free hydrocyanic acid is slowly converted into other substances by these enzymes[5] and if the same process occurred with mercuric cyanide it would influence the preservative efficiency of mercuric chloride with cyanogenetic plants.

A solution of mercuric cyanide in water was adjusted to contain 21.2 mg. of hydrocyanic acid per 25 cc. To 1 liter of this solution was added 13.3 gm. of an active dhurrinase preparation from sorghum; to 1 liter, 13.3 gm. of an active prunase preparation from *Prunus melanocarpa*. A third liter was kept under the same conditions as a control. The solutions were kept in the dark at 25° C. in pyrex flasks with rubber stoppers. At intervals 25-cc. portions were withdrawn and the hydrocyanic acid content was determined. The mercuric

[5] COUCH, JAMES F., and BRIESE, REINHOLD, R. THE DESTRUCTION OF HYDROCYANIC ACID BY PRUNASE AND THE INFLUENCE OF SUGARS ON THE REACTION. Wash Acad. Sci. Jour. 29: 219–221, illus. 1939.

cyanide was decomposed with stannous chloride and the freed acid was distilled into 5-percent sodium hydroxide, the distillate being then titrated according to the Liebig-Denigès method. The results are presented in table 10. At the end of 15½ weeks each solution had lost 2.8 percent of hydrocyanic acid. It was evident that the cyanogenetic enzymes did not hasten decomposition of mercuric cyanide under the conditions of this experiment.

TABLE 10.—*Stability of mercuric cyanide solutions at 25° C. in the presence of cyanogenetic enzymes*

Time of storage (weeks)	Hydrocyanic acid recovered per 25 cc. of solution			Time of storage (weeks)	Hydrocyanic acid recovered per 25 cc. of solution		
	Control	With dhurrinase	With prunase		Control	With dhurrinase	With prunase
	Milligrams	*Milligrams*	*Milligrams*		*Milligrams*	*Milligrams*	*Milligrams*
0	[1] 21.2	[2] 21.2	[3] 21.1	5	20.7	20.7	20.7
1	21.1	20.9	20.5	6	20.8	20.5	20.7
2	21.2	20.9	20.7	10	20.9	20.7	20.6
3	20.8	20.9	20.8	15½	20.6	20.6	20.5
4	20.8	20.8	20.7				

[1] pH 6.10, at end 6.50.
[2] pH 6.67, at end 5.79.
[3] pH 6.32, at end 5.19.

OTHER MERCURY COMPOUNDS AS PRESERVATIVES

One set of experiments was made to determine the comparative efficiency of mercuric chloride, mercuric oxide, and mercurous chloride as preservatives. A collection of fresh hegari leaves was preserved in the usual manner with each of these compounds in a concentration of 1 percent based on the green weight of the plant. The samples were analyzed after 1, 4, and 6 months. The results are presented in table 11. Neither of the other two compounds tested was so efficient as mercuric chloride. After 1 month the mercuric oxide sample had lost 20 percent and after 4 months the mercurous chloride sample had lost over 15 percent. At the end of 6 months these samples had lost still more, whereas the mercuric chloride sample had nost none. In all three samples the quantity of mercury present was much in excess of that needed to combine with all the hydrocyanic acid present.

TABLE 11.—*Hydrocyanic acid recovered from fresh hegari macerated for various periods with different mercury compounds*

Period of preservation (months)	Hydrocyanic acid per 100 gm. of dry plant after maceration with 1 percent of—		
	Mercuric chloride	Mercurous chloride	Mercuric oxide
	Milligrams	*Milligrams*	*Milligrams*
1	35	35	28
4	36	29	26
6	35	26	24

INFLUENCE OF HYDROGEN-ION CONCENTRATION ON PRESERVATION

Cyanogenetic plants develop their maximum content of hydrocyanic acid under certain optimum conditions of acidity.[6] It was therefore of interest to determine the influence of acidity on the development of the hydrocyanic acid during preservation. Samples preserved in the usual way were used; results are presented in table 12. It was found that the increased acidity due to the mercuric chloride was beneficial, the average pH of the samples falling within the optimum range. No evidence was obtained that hydrochloric acid derived from the mercuric chloride was formed in excess or repressed cyanogenesis in the mixtures.

TABLE 12.—*Hydrocyanic acid content and pH of samples of hegari macerated with and without the use of mercuric chloride*

Collection No.	Mercuric chloride used [1]	Time of maceration	pH of sample			Hydrocyanic acid recovered per 100 gm. of plant
			At beginning	At end	Average	
	Percent	*Days*				*Milligrams*
29–38	0	1	5.63	4.74	5.19	171
62–38	0	1	5.86	4.19	5.03	123
62–38	1	28	5.28	5.34	5.31	159
62–38	2	56	5.05	4.14	4.6	193
95–38 [2]	0	1	5.47	3.92	4.7	288
95–38	1	56	5.19	4.23	4.71	367
95–38	2	56	5.07	3.99	4.53	342

[1] Calculated on weight of plant.
[2] Second-growth, all others first growth.

SUMMARY AND CONCLUSIONS

A further study was made of the mercuric chloride method for preserving cyanogenetic plants for chemical analysis. Experiments were made with 14 varieties of sorghum, 2 species of wild cherry, and 1 species of arrowgrass. This study extends and confirms the results previously reported.

On the whole, good preservation was obtained for periods up to 6 months and, in a number of cases, for 1, 1½, and 2 years. Young plants are more difficult to preserve than older plants. This is due to their higher content of hydrocyanic acid, to the rapidity with which it is developed in bruised tissues, and also to the fact that the cyanogenetic enzyme appears to be much more susceptible to the inhibiting action of mercuric chloride than is the case in older tissues.

A concentration of 1 percent of mercuric chloride based on the weight of the plant sample is sufficient to preserve specimens that contain 100 to 125 mg. of hydrocyanic acid per 100 gm. Specimens that contain larger quantities of the acid require 2 to 3 percent of mercuric chloride for satisfactory preservation. Concentrations larger than 3 percent offer no advantages but have the disadvantage of markedly inhibiting cyanogenesis.

The rate of cyanogenesis in the plants studied when they were preserved in 1 percent of mercuric chloride is such that in general the

[6] COUCH, JAMES F., and BRIESE, REINHOLD R. HYDROGEN ION CONCENTRATION AND CYANOGENESIS IN SORGHUM. Amer. Jour. Pharm. 111: 55–64, 151–160, 193–201. 1939.

maximum amount of hydrocyanic acid is liberated in 3 to 8 weeks. With higher concentrations of the mercury compound the maximum quantity may not be attained for 6 to 12 months.

The rate of cyanogenesis in the preserved samples could not be regularly increased by the addition of the appropriate enzyme to the sample.

The cyanogenetic enzymes dhurrinase and prunase did not affect the stability of a solution of mercuric cyanide over a period of 15½ weeks.

Mercuric oxide and mercurous chloride were less efficient as preservatives than mercuric chloride.

The hydrogen-ion concentration of mixtures of sorghum and mercuric chloride falls within the optimum pH range for cyanogenesis in sorghum. The addition of mercuric chloride may be regarded as beneficial from this standpoint.

Figures for the hydrocyanic acid obtained from samples of plants macerated in water, except for a few irregular cases, fall much below those obtained by the use of mercuric chloride. Water maceration cannot be relied upon to furnish accurate data for the hydrocyanic acid content of cyanogenetic plants.

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